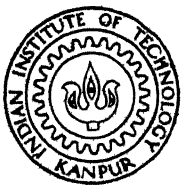
INTERACTION OF A/(III) WITH PROTEINS

by
D. D. KUTTE



DEPARTMENT OF CIVIL ENGINEERING
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INTERACTION OF A/(III) WITH PROTEINS

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for the Degree of
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D. D. KUTTE

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CERTIFICATE

This is to certify that the present work titled, "INTERACTION OF Al(III) WITH PROTEINS" has been carried out by Shri D.D. Kutte under my supervision and the same has not been submitted elsewhere for a degree.

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D.D. KUTTE

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ABSTRACT

An attempt has been made to investigate the interaction of proteins with aluminium ions. The experiments have been performed to study two aspects namely:

(i) hydrolysis of aluminium, and

(ii) aluminium-protein interaction.

Hydrolysis of aluminium sulphate leads to early precipitate formation and precipitation is almost complete at pH 4.5. Constitution of precipitate was found to be $Al(OH)_{2.4}(SO_4)_{0.3}$, upto pH 4.5, and it gradually varied to $Al(OH)_3$ with increase in pH. Increase in SO_4^{--} ion concentration in the solution has no effect on hydrolysis equilibrium. Similarly, change in ionic medium did not affect the aluminium hydrolysis.

In the aluminium-protein interaction experiments, two proteins were used; (1) Bovine Serum Albumin, and (2) Casein. Casein showed greater binding of aluminium ions in comparison to bovine serum albumin. The amount of aluminium bound to casein molecules remained constant upto pH 8.0, whereas in case of bovine serum albumin it reduced with the increase in pH value. Presence of calcium increased the amount of proteins precipitated. This was true in case of casein, but calcium did not have any effect on precipitation of albumin. Diprotic acids like oxalic

acid and malic acid reduced the amount of aluminium bound to bovine serum albumin and showed marginal effect in case of aluminium binding on casein.

CHAPTER I

INTRODUCTION

In view of the need for better and reliable water quality control and the necessity of wastewater reuse, there has been a continuous interest in physico-chemical processes for wastewater treatment. Several authors have recently reported new summaries on pilot plant studies and recommended strongly direct physico-chemical treatments, as an alternative to the biological tertiary process scheme, with complete elimination of biotreatments (Weber, 1970; Kreissl et al., 1972; Kreissl et al., 1973). The advantage of P-C processes is their easy and reliable controllability, insensitivity to transients, and to toxicants.

Physico-chemical processes incorporate, in general, following unit operations,

(1) Chemical flocculation and clarification,

(2) Filtration, and

(3) Adsorption through activated carbon column.

The above mentioned, as well as other studies reported in recent years on direct P-C treatments, contribute to the advantage of P-C processes but lack in detailed information pertaining to the removal of specific organics

and inorganics associated with the above mentioned unit operations. This happens to be due to the characteristics of wastewater used for the investigation. The studies were done with very weak wastewaters, and therefore from efficiency point of view result of organics were reported in terms of general parameters such as TOC, COD, and BOD. Wastewater characteristics are dependent on economic, as well as social background. In developing countries because of lower per capita water consumption the wastewaters are much stronger in character. Rebhun and Streit (1974) investigated direct physico-chemical treatment of strong raw wastewaters. They found out that not only the suspended and most colloidal fraction of the organic compounds were efficiently removed, but considerable fraction of the 'solubles' was also removed, particularly of the proteins by chemical flocculation and clarification of raw wastewaters. In the opinion of above authors, the affected 'solubles' were probably high molecular weight substances or macromolecules behaving as hydrophilic colloids and were removed by chemical reaction, adsorption, and flocculation. In the above mentioned investigation, chemicals used in carrying out chemical flocculation were, ferric chloride and calcium hydroxide. Use of alum for coagulation and flocculation of wastewaters is not uncommon. Ulmgreen (1974) has reported the use of alum in numerous wastewater treatment plants in Sweden.

Precipitation, due to addition of trivalent cations like aluminium, of macromolecules like proteins in chemical flocculation units needs a greater attention. If it is possible to aggregate the macromolecules in the very first unit operation of P-C processes, then the loading of solubles on polishing units like adsorption columns will be automatically maintained at minimum possible level. This is important from economy point of view, as adsorption columns with the use of activated carbon involve considerable investment in comparison to the cost of chemicals used. Study of interaction of macromolecules with metal ions may provide a potential insight to the multiple benefits gained with the use of coagulants like alum, ferric chloride etc.

Macromolecules belong to the realm of Colloid Science. Interaction of macromolecules like proteins with metal ions will be very well understood, with the concept of their stability in the solutions. Hardly any data is available pertaining to interaction of organic colloids with metal ions like aluminium, in comparison to inorganic colloids.

The present work was undertaken to delineate the basic mechanism involved in the precipitation or aggregation of macromolecules like proteins by chemical coagulation.

Various parameters, believed to affect the process were also evaluated. It is believed that the information obtained from this investigation, will contribute to basic knowledge regarding precipitation of proteins from wastewaters by chemical coagulation and flocculation.

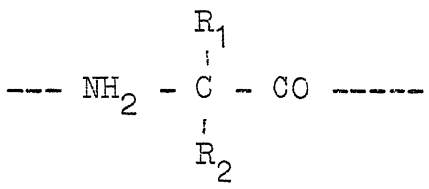
CHAPTER II

LITERATURE SURVEY

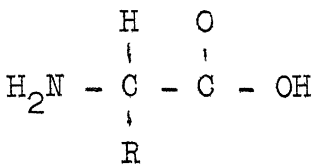
2.1 Proteins

2.1.1 Chemical Composition and Structure:

The proteins are characterised by containing main chains, built up from the structural unit



Here R_1 and R_2 in the simple cases are H-atoms only, but they may be extended to complicated groups, which are referred to as side chains. All these proteins can be broken down by hydrolysis to yield organic molecules, all of which are amino acids. These amino acids, furthermore all have the structure



i.e. they are α-amino acids, the name signifies that the NH_2 group is attached to the carbon atom adjacent to the acid function. These α-amino acids are distinguished by different R groups.

In structural studies it is customary to distinguish between fibrous and globular proteins. The fibrous proteins tend to occur as long chains, and are found in structural tissues such as wool, muscle etc. The globular proteins, on the other hand tend to be more or less spherical and, unlike the fibrous proteins, are often water soluble.

The basic element of protein structure are essentially polypeptide chains. The structure of proteins can be examined at four different levels: the primary, secondary, tertiary and quarternary structure. The primary structure is the sequence of the amino acid residues in the polypeptide chains. The secondary structure refers to the way in which polypeptide chain is coiled as a sequence of the links and bonds between relatively close elements of the chain. The tertiary structure describes how the more or less coiled polypeptide chain are arranged in space, folded or packed into the protein molecule by side chain interaction, and cross-linked by disulphide bridge. The quaternary structure has been introduced in order to account for the level of organization in which complex molecules are formed by association of macromolecular units.

The side chains 'R' on amino acids are the cause of amphoteric behaviour of proteins. Soluble protein molecules are found in highly hydrated state. The charge

and solvated water layer constitute to stability of protein molecules in solution. The charge on protein molecules, thus depends on the degree of ionization of polar groups on the side chains.

2.2 Interaction of Cations with Proteins

Interaction of proteins with inorganic cations play a significant role, in deciding their stability in solution. Joly, and Barbu (1950) observed aggregation of Horse Serum Albumin in the presence of aluminium chloride at room temperature. They found out that these aggregates do not precipitate but remain in suspension: The concentration of aluminium was of the order of $4x10^{-4}$ M. Non settling nature of aggregates indicate the presence of considerable amount of bound water. Salahuddin, and Malik (1964) studied the interaction between aluminium and gelatin. They concluded that the carboxylic groups of the proteins made themselves available for combination with metal ions. Salahuddin (1965) has observed that aluminium ions react with casein molecules between pH values 3 to 6.5. Most probable site of interaction seems to be carboxylic group on the casein molecule. Similar was the observation of Salahuddin and Malik (1962), while investigating the interaction between alumina solutions and

gelatin, in a narrow pH range from 3.7 to 5. This shows that interaction of aluminium with different proteins is probably limited to the carboxyl group only.

No quantitative data seems to be available on aluminium interaction with proteins, resulting into aggregation of protein molecules from the solution. Considerable work has been done regarding interaction of aluminium ions with virus proteins. Lal, and Ebba Lund (1974), precipitated virus with the use of aluminium sulphate. They observed good recovery of viruses in precipitate. The precipitation was followed by eluation with the help of phosphate buffer. But even after phosphate buffer treatment, aluminium precipitate still contained an important amount of virus. Virus strain used in above experimentation was Coxsackie B_3. Chaudhuri (1969) studied the kinetics of adsorption of aluminium on bacteriophages. In case of Bacteriophage MS2, amount of aluminium adsorbed remained almost constant, and was found to be independent of pH. But the amount of aluminium adsorbed on Bacteriophage T_4 decreased with the pH. He has concluded that interaction between aluminium and virus coat proteins result possibly in the formation of co-ordination complex between aluminium and carboxyl group of virus protein. Adsorption of aluminium on Bacteriophage T_4 follows Langmuir

adsorption equation in the above mentioned investigation. This leads to the conclusion that all sites on the virus coat protein are energetically similar, and therefore aluminium forms only one type of co-ordination complex at the interface. The amount of aluminium adsorbed in case of T_4 virus is almost equivalent to number of carboxylic groups on the virus protein coat. This suggest the possibility that only one type of complex exists in the pH range 5-9. Aluminium ions show considerably different hydrolysis products in the pH range 5-9. Therefore aluminium coordination complex at the interface must be stable than $Al(OH)_4^-$ species normally found at high pH values. This may suggest that aluminium ion may be forming co-ordination complex with more than one carboxyl group on the protein coat. But observation indicates that number of aluminium ions are almost equal to number of carboxyl groups. Adsorption is found to be instantaneous reaction. This indicates that the mechanism of aluminium binding to protein coat seems to be quite complicated one.

At higher pH values, the interaction between proteins and aluminium seems to be different, than that at low pH values. Hydrophobic colloids also show instantaneous adsorption of polynuclear hydroxo complexes of aluminium, but normally concentration of alum added is

quite high. It is necessary to catagories properties of different functional groups present on protein molecules and their interaction with metal ions like aluminium. This may provide a better understanding of interaction between metal ions and macromolecules like protein.

2.3 Properties of Functional Groups

The marked difference in the coagulative response of carboxyl, sulphate, and phosphate colloids towards a given multivalent cation and the different sequence of cations, will regard to the relative position of their critical concentration for each type of colloids is explainable if one considers the great variation in the affinity of inorganic cations towards the different functional groups. For example, 10 to 100 times smaller concentration of Pb, Cd, Ca, and Zn are needed for coagulation of phosphate colloids than for coagulation of carboxylic colloids (Bungenberg De Jong, 1949). LaMer and Smellie (1956) found out that potato starch, which contains phosphoryl monoester, is a most effective coagulant for colloidal ore dispersion, whereas corn starch which contains no phosphate easter is ineffective. By using distilled water, it was further shown that flocculation of potato starch was possible only when cations that form insoluble phosphates

were present. Wettstein et al. (1961) studied complex formation equilibrium of crossed linked starch phosphates with various cations. An increase in complexing tendency was observed in the following order $K < Ca < Zn < Fe^{3+}$.

From the above observations it can be concluded that the interaction of inorganic cations with macromolecular colloids like carbohydrates, proteins will be controlled by the functional groups on the subsequent molecules.

2.4 Complexes of Aluminium

The chemical co-ordination of polyvalent metal ions to the ionised groups of hydrophilic colloids is of considerable significance in flocculation reaction between coagulant metal ions and hydrophilic colloids. Al(III) has strong tendency to form soluble and insoluble complexes with hydroxide ions. Because complex formation in solution consists of replacement of water molecules in solvated shell of the free metal ion by other ligands (Bjerrum et al., 1958), it should be recognised that OH^- ion is simply one among many ligands which might react with metal ion. Complex formation occurs not only with OH^- ions but with other bases (Sillen, Martell, 1972 ; Lacroix, 1948). Aluminium forms complexes with substances carrying

carboxylic or hydroxyl functional groups, as well as inorganic phosphates (Hsu, 1968), and to minor extent sulphates and chlorides (Hsu, Bates, 1964). Stumm, and Morgan (1962) performed alkalimetric titrations with aluminium ions in the presence of phosphates, pyrophosphates, salicylate, and oxalate ions, results indicate that these ions form both soluble complexes and insoluble precipitates with aluminium ions. This complexing tendency of aluminium ions towards different bases exhibits its potentiality in coagulating hydrophobic as well as hydrophilic colloids from water and wastewaters, perhaps this may be the reason for its utility in most of the water and wastewater treatment plants.

CHAPTER III

THEORY

Stability constants of a few polynuclear halides, polyacids, and other hydroxo complexes were reported at the beginning of the twentieth century (Sherril, 1907). More work was done in this field by Pyrtz (1931) some twenty years later, but a further gap of twenty years ensued before rigorous, quantitative studies of polynuclear complexes B_qA_p became more common. Chemists have tended to neglect the formation of polynuclear complexes and to study simpler mononuclear equilibria. When it was proved impossible to interpret some particular system solely, in terms of mononuclear species, the existance of one or two polynuclear species has often been postulated in an arbitrary fashion (Ahrland, et.al., 1954; Sillen, 1954). Consequently, both the identification of these species and the reported values of the stability constants are usually unreliable. Although it is possible to interpret some polynuclear equilibria rigorously in terms of the existence of only a few complexes, in many cases it is necessary to postulate the existence of a series of complexes.

In the present investigation, system under study, consisted of Al^{3+}-SO_4^{--}-OH^--H_2O. Information regarding

distribution of polynuclear complexes and their stability constants can be found out by using the following rigorous mathematical procedure.

3.1 General Approach

Reaction between metal ion and ligand can be expressed in a generalised form in the following manner,

$$qB + pA \rightleftharpoons A_pB_q \qquad (1)$$

where, P - Symbolizes metal ion, and

A - Ligand.

Let free concentration of A = a, and that of B = b and concentration of A_pB_q = C_{qp}

$$\therefore \text{ Equilibrium constant } B_{qp} = \frac{A_pB_q}{b^q a^p} \qquad (2)$$

$$= \frac{C_{qp}}{b^q a^p} \qquad (2a)$$

In particular, hydrolysis reaction can be written in a similar fashion,

$$qB + pH_2O \rightleftharpoons B_q(OH)_p + pH^+ \qquad (3)$$

The above equations can be conveniently expressed in terms of hydrolysis constants

$$x_{qp} = \frac{B_q(OH)_p \; h^p}{b^q} = B_{qp} \cdot Kw^p \qquad (4)$$

$Kw = [H^+] [OH^-]$

= dissociation constant

h = equilibrium hydrogen ion concentration.

where 'B_{pq}' is defined by equation (2), with hydroxyl ion as a ligand. The total concentration of central group or metal ion and ligand respectively are given by

$$B = \sum_{1}^{Q} \sum_{0}^{P} q(B_qA_p) = \sum_{1}^{Q} \sum_{0}^{P} q.C_{qp} \qquad (5a)$$

and $$A = a + \sum_{1}^{Q} \sum_{1}^{P} p(B_qA_p) = \sum_{1}^{Q} \sum_{1}^{P} p.C_{qp} \qquad (5b)$$

where P and Q are maximum values of p and q respectively.

For simplicity in analysis, following assumptions can be made:

i) Polymerization of A, i.e. OH^-, and competetive complex formation (e.g. with protons i.e. H^+ ions) are ignored.

ii) SO_4^{--} ions do not compete with OH^- ions, while forming complex with metal ions.

It is convenient to define two secondary concentration variables in the same way as for mononuclear systems. Combining equations (5a, b), the average number of ligands bound to central group is

$$\bar{n} = \frac{A - a}{B} = \sum_{1}^{Q} \sum_{1}^{P} p.C_{qp} \Big/ \sum_{1}^{Q} \sum_{0}^{P} q.C_{qp} \qquad (6)$$

and rearranging equation (5a), the ratio of total to free metal ion concentration is

$$F_o = \frac{B}{b} = \sum_{1}^{Q} \sum_{0}^{P} q.B_{qp}.b^{q-1}.a^{p} \qquad (7)$$

The polynuclear hydrolysis of metal ions is most conveniently treated by expressing the average number of hydroxyl ions bound to each metal ion as a polynomial in h^{-1}, the equation (6) is replaced by

$$\bar{n} = \frac{A - a}{B} = \frac{h - H - Kw.h^{-1}}{B} \qquad (8a)$$

$$= \frac{\sum_{1}^{Q} \sum_{1}^{P} p.x_{qp}.b^{q}h^{-p}}{\sum_{1}^{Q} \sum_{0}^{P} q.x_{qp}.b^{q}h^{-p}} \qquad (8b)$$

where, h - equilibrium hydrogen ion concentration

Kw - dissociation constant for water

H - total analytical excess of hydrogen ion concentration, and x_{pq} and h^{-1} replace B_{pq} and 'a' respectively in subsequent equation. In generalised equation A is replaced by H and a by 'h^{-1}'. The hydrocomplexes, instead of B_qA_p, are represented as (B_qH_{-p}).

If $h < 10^{-5}$ M, the equation (8a) is written as

$$\bar{n} = \frac{A - a}{B} = \frac{h - H}{B} \qquad (8c)$$

In general, $\bar{n}$ and F_o are functions not only of (h^{-1}) but also of 'b', and hence, of total metal ion concentration B. Both $\bar{n}(h^{-1})$ and $F_o(h^{-1})$ can be calculated directly by using equation (5a) and (5b), for a series of values of B, for those systems in which corresponding values of A, B, a, b can be measured, this has been done, e.g. in potentiometric study of the hydrolysis of Uranium (Hietanen, 1956), Iron (III) (Hedstrom, 1953), Indium (Bececki-Biedermann, 1956).

More often, however, it is possible to measure only A, B, a i.e., H, B, h^{-1} from which $\bar{n}$, but not F_o, can be calculated directly.

3.2 Calculation of Free Concentration of Central Groups and Ligand

It is possible, however, to calculate b from data B, H, h without making any assumptions about the nature of complexes. It follows from equation (5a) and (5b)

$$\left(\frac{B}{\partial \ln h}\right)_{b=\text{const.}} = \left(\frac{H}{b}\right)_{a=\text{const.}} \qquad (9)$$

which can be expressed in Jacobian-functional notation as

$$J(B, \frac{\ln b}{\ln h}, A) = \frac{\partial (B, \ln b)}{\partial (H, \ln h)} = 1 \qquad (10)$$

Since any pair of B, H, n^{-1} and b can be used as independent variables. Integration of equation (10) with B and H as independent variables gives the Hedstrom-McKay equation (Hedstrom, 1955; McKay, 1953).

$$- \log F_o = \log \frac{b}{B} = \left[\int_0^H \left(\frac{\log \frac{h^{-1}}{H}}{B} \right)_A .dA \right]_B \qquad (11)$$

and

$$\log \frac{h^{-1}}{H} = \left[\int_0^B \left(\frac{\log \frac{b}{B}}{A} \right)_B .dA \right]_H \qquad (12)$$

Hedstrom used equation (12) to calculate values of 'h', the free hydrogen ion concentration from data B, H, b in the hydroxo-iron system, and showed that they were in good agreement, as compared to the measured ones.

The calculation is more convenient by Sillen's method (1961), which requires fewer data, B, H, h, and does not need an auxiliary plot. The form of equation (10) with B and $\ln h^{-1}$ as independent variables is

$$\left(\frac{\ln B}{\ln h^{-1}} \right)_{B=C} = - \left(\frac{\partial H}{\partial B} \right)_{h^{-1}=C} \qquad (13)$$

whence by combination of equations (6) and (7)

$$\log F_o = \log \frac{B}{b} = -\int_0^a \left[\bar{n} + \left(\frac{\bar{n}}{\ln B}\right)_{n'} \right] d \log h^{-1} \Big\}_B \qquad (14)$$

for systems mononuclear at the lower limit of integration.

3.3 Average Composition of Complex

Values of $\bar{n}$ are usually less informative in polynuclear systems than in mononuclear systems. However, if B, H, b and h are all known, it is possible to calculate the quantity

$$\bar{W} = \frac{h - H - Kw.h^{-1}}{B - b} = \sum_1^Q \sum_1^P p.x_{qp}.b^q h^{-p} \Big/ \sum_1^Q \sum_0^P q.x_{qp}.b^q h^{-p} - b \qquad (15a)$$

$$= \frac{\bar{p}}{\bar{q}} \qquad (15b)$$

where, $\bar{p}$ and $\bar{q}$ are the average numbers per complex of ligand and metal ion respectively. It is possible to calculate $\bar{p}$ and $\bar{q}$ separately without making any assumptions about the nature of complexes. The mean degree of condensation of all species containing B is given by Sillen (1961)

$$\frac{1}{\bar{r}} = \frac{\sum_1^Q \sum_0^P q.x_{qp}.(b)^q(h)^{-p}}{\sum_1^Q \sum_0^P p.x_{qp}.(b)^q(h)^{-p}} = \frac{B}{\sum_1^Q \sum_0^P C_{qp}} \qquad (16)$$

Combining equations (6), (15), and (16),

$$\bar{p} = \frac{\sum_{1}^{Q}\sum_{1}^{P} p\, x_{qp}(b)^{q}(h)^{-p}}{\sum_{1}^{Q}\sum_{0}^{P} C_{qp} - b}$$

$$= \frac{h - H}{B\bar{r} - b} \tag{17a}$$

$$= \frac{B\bar{n}}{B\bar{r} - b} \quad .$$

$$\bar{q} = \frac{B - b}{B\bar{r} - b} \tag{17b}$$

Method for calculating $\bar{r}$ has been derived by Sillen (1961). Differentiation of equation (16) and combination with equations (5a), (5b), and (6) give

$$d(B\bar{r}) = Bd\bar{r} + \bar{r}\, dB$$

$$= B\bar{n}\, d\, \ln h^{-1} + B\, d\, \ln b \tag{18}$$

whence, $d(\ln b - \bar{r}) = \bar{r}\, d\, \ln B - \bar{n}\, d\, \ln h^{-1}$

$$d(\ln b - \bar{r}) = \bar{r}\, d\, \ln B - \bar{n}\, d\, \ln h^{-1} \tag{19}$$

and thus,

$$\left(\frac{\partial \bar{r}}{\partial \ln h^{-1}}\right)_{B=\text{const.}} = -\left(\frac{\partial \bar{n}}{\partial \ln B}\right)_{h^{-1}=\text{const.}}$$

which may be expressed in Jacobian-functional notation as

$$J\left(\ln B,\ \bar{r}/\ln h^{-1},\ \bar{n}\right) = \frac{\partial(\ln B,\ \bar{r})}{\partial(\ln h^{-1},\ \bar{n})} = 1 \tag{20}$$

where, any pair of B, h^{-1}, $\bar{n}$, and $\bar{r}$ can be treated as independent variables. Integration of equation (20) gives, with h^{-1} and B as independent variables

$$\bar{r} = r_1 - \left[\int_0^a \left(\frac{\bar{n}}{\log B}\right)_{h^{-1}} d \log h^{-1}\right]_B \qquad (21)$$

Similarly, with $\bar{n}$ and B as independent variables,

$$\bar{r} = r_1 + \left[\int_0^{h^{-1}} \left(\frac{\log h^{-1}}{\log B}\right)_{\bar{n}} d\bar{n}\right]_B \qquad (22)$$

when $\bar{r}$ is known, it is possible (Sillen, 1961) to calculate b by combining equations (19), and (22), obtaining

$$\log \frac{B}{b} = \left[\int_0^a \bar{n}.d \log h^{-1}\right]_B + 1 - \bar{r} \qquad (23)$$

3.4 Elimination of Mononuclear Terms

A more reliable indication of the identities of polynuclear complexes may be obtained if it is possible to eliminate the mononuclear terms from $\bar{p}$, $\bar{q}$, and other terms (Sillen, 1961). It is convenient to break up a number of the primary and secondary concentration variables into separate terms for each value of q(Ingri, 1959; Rossotti et al., 1956). Thus,

$$B = \sum_1^Q B_q = B_1 + B_n \qquad (24)$$

where, B_1 - concentration of metal ions in mononuclear form

B_n - concentration of metal ions in polynuclear complex

B - total metal ions added

$$B_q = q \sum_{0}^{P} B_q A_p$$

$$= qb^q \sum x_{q,p} . h^{-p}$$

$$= qb^q f_q \qquad (25)$$

and f_q is a function of h^{-1}. Similarly,

$$B\bar{n} = \sum_{1}^{Q} B_q \bar{n}_q$$

$$= B_1 \bar{n}_1 + B_n \bar{n}_n \qquad (26a)$$

where $$B_q \bar{n}_q = \sum_{1}^{P} p\, B_q A_p$$

$$= b^q \sum_{1}^{P} p\, x_{q,p}\, h^{-p}$$

$$= qb^q f_q \bar{n}_q \qquad (26b)$$

and $\bar{n}_q$ is also a function of h^{-1}. Moreover

$$B\bar{r} = \sum_{1}^{Q} B_q r_q = B_1 + B_n \bar{r}_n \qquad (27)$$

where $B_q r_q = \sum_{0}^{P} B_q A_p$

$$= \frac{B_q}{q} \tag{28}$$

Combining equations (6), (24), and (26a)

$$\bar{n} = \frac{\bar{p}}{\bar{q}} = \frac{B\bar{n} - B_1\bar{n}_1}{B - B_1}$$

$$= \frac{\sum_{2}^{Q} \sum_{1}^{P} p\, x_{qp}\, b^{q}h^{-p}}{\sum_{2}^{Q} \sum_{0}^{P} q\, x_{qp}\, b^{q}h^{-p}} \tag{29}$$

where $\bar{p}$ and $\bar{q}$ are the average numbers of A and B, respectively, in each polymer complex. Combining equations (24), and (27),

$$\frac{1}{\bar{r}_{\pi}} = \bar{q}_{\pi} = \frac{B - B_1}{B\bar{r} - B_1} \tag{30}$$

whence, by combination with equation (29),

$$\bar{p} = \frac{B\bar{n} - B_1\bar{n}_1}{B\bar{r} - B_1} \tag{31}$$

when $\bar{r}$ has been determined, all terms required for the calculation of $\bar{p}_{\pi}$ and $\bar{q}_{\pi}$ from equations (30), and (31) will be known except B_1 and $\bar{n}_1$. However, in many polynuclear systems, the function $\bar{n}(\log h^{-1})B$ is independent of B below

some limiting value of B and can be assumed to be $\bar{n}_1(\log h^{-1})$. Alternatively, it is possible to obtain $\bar{n}_1(\log h^{-1})$ by extrapolation (Sonesson, 1958 and 1960). In general

$$\lim_{B \to 0} \bar{n} = \bar{n}_1$$

and it is usual to extrapolate the functions $\bar{n}(B)h^{-1}$.

If it appears from the calculated values of $\bar{p}$ and $\bar{q}$ that only two or three complexes are formed, normalized curves may be calculated (Sillen, 1956) on the assumption that certain species coexist. In this way it is possible to discover which species are probably present and to determine the relevant stability constants.

CHAPTER IV

SCOPE OF INVESTIGATION

From the preceding discussion, it is evident that there is a great need to obtain more information on the interaction of Al(III) with soluble proteins which results in their precipitation. It is essential to know the parameters, which control the segregation of protein molecules from solution, so as to understand the limitations involved pertaining to efficiency of chemical flocculation and clarification, a unit operation in physico-chemical processes of wastewater treatment.

The precipitation of proteins in treatment plants, due to addition of alum, is till now a mere observation. Aluminium ions do not play a predominant role in living systems. Perhaps it may be the reason that hardly any data is available regarding the interaction of Al(III) ions with soluble protein molecules, in comparison to ions like copper, zinc, mercury etc.

By reference to the observations, pertaining to Al-protein systems, protein precipitation or aggregation mechanism can be postulated as follows:

(1) Chemical reaction takes place between Al(III) ions and carboxylic groups on the protein molecule. This

may cause reduction in interparticle repulsion and cause aggregation of protein molecules.

(2) Al(III) ions and its polynuclear hydroxo complexes may interact in similar way as they do with inorganic colloids, and thus causing flocculation of protein molecules.

(3) Structural break up, causing unfolding of protein chain. These unfolded chains, because of interlocking may result in aggregation of protein molecules.

Before investigating about Al-protein interaction, it is essential to study Al hydrolysis in detail. Equilibrium constants for Al(III) hydrolysis were found out from pure systems in which anions like SO_4^{--} were not present. Detailed study of Al-SO_4 hydrolysis would help in understanding Al-protein interaction in a more precise manner. Therefore, the present investigation was carried out in two phases:

(I) Study of Al-SO_4 hydrolysis at ionic strength = 0.01 M to find out:

(i) distribution of hydrolytic species at different equilibrium pH values,

(ii) kinetics of hydrolysis,

(iii) effect of SO_4^{--} ion on the equilibrium distribution of hydrolytic species, and

(iv) effect of ionic medium maintaining ionic strength at 0.01 M on aluminium hydrolysis.

(II) Study of Al-protein interaction to find out:

(i) equilibrium concentration of soluble and aggregated proteins at different Al(III) and protein concentrations,

(ii) kinetics,

The effect of following variables in protein aggregation by Al(III) were also investigated:

(iii) pH,

(iv) bivalent cations like calcium and magnesium, and

(v) diprotic acids like oxalic, and malic acid.

CHAPTER V

METHODS AND PROCEDURE

5.1 Methods

5.1.1 Determination of Aluminium:

A simple, rapid and sensitive method for determining soluble aluminium concentration in the microgram range was required. The erichrome cyanine R method originally introduced by Knight (1960) and later modified by Shull and Guthan (1967), and Chaudhuri and Engelbrecht (1968) was used. Instead of erichrome cyanine R, solochrome cyanine R was used as suggested by Standard Methods (1971). The standard aluminium curve shown in Figure 1, was used to determine the aluminium concentration in unknown samples.

In the procedure 10 to 25 ml sample was used. This sample was first titrated to pH 4.0 with N/50 sulphuric acid and one ml was added in excess. This was followed by the addition of one ml of ascorbic acid (0.19/100 ml) and 10 ml of buffer solution (136 gm sodium acetate plus 40 ml of 1.0 M acetic acid and double distilled water to final volume of one litre). Finally 5 ml of 0.03 percent solochrome cyanine R was added, mixed and immediately made up to a

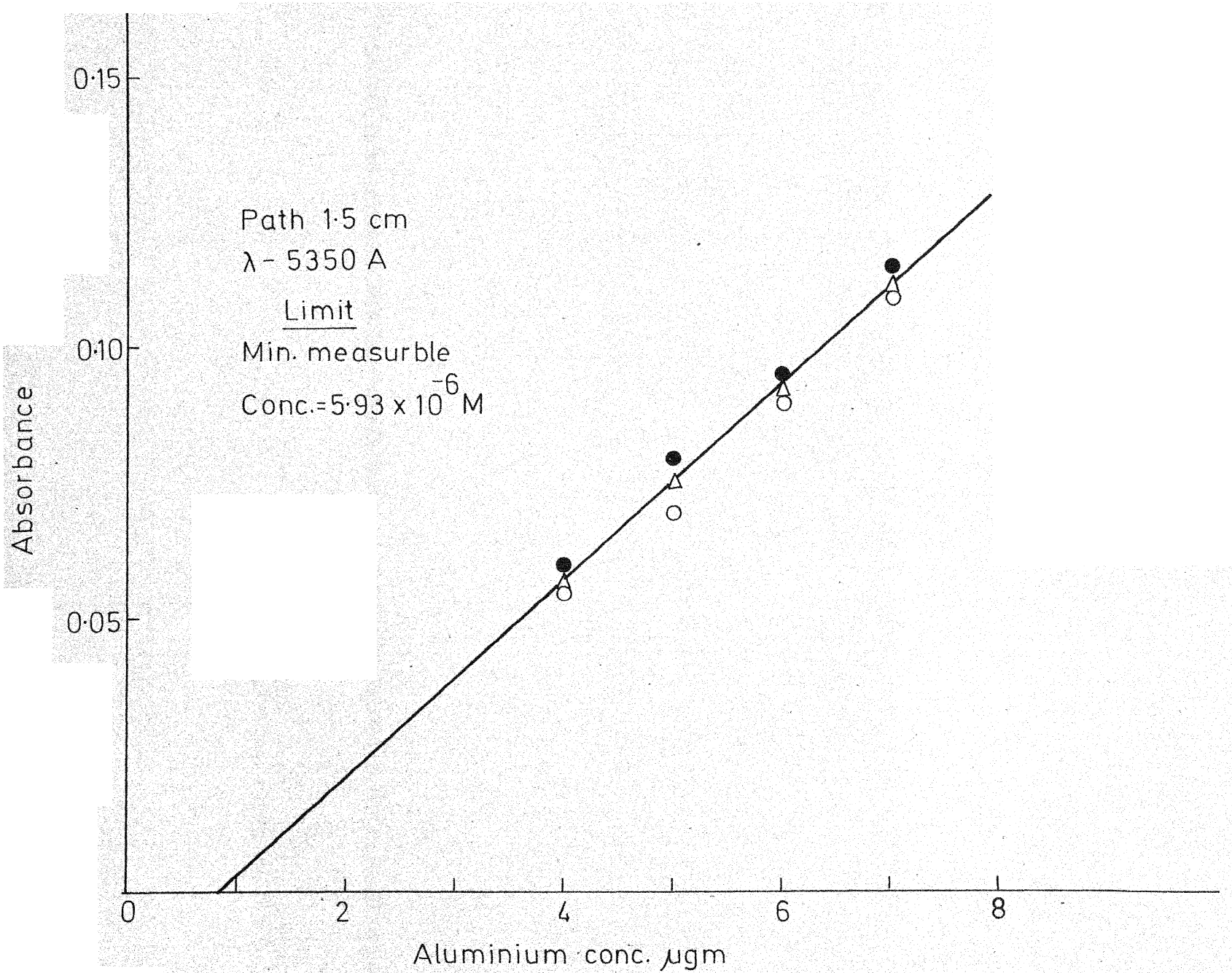


FIG. 1 STANDARD CURVE FOR ALUMINIUM

volume of 50 ml with double distilled water. The sample was mixed again and allowed to stand for 5 to 10 minutes. The absorbance was read against a suitable blank on spectrophotometer (Spectronic-20, Bausch and Lomb, U.S.A.) using a wavelength of 535 mμ and a 1.5 cm light path. All readings were corrected for dilution due to added solutions. For plotting calibration curve, the procedure was repeated 4 time to know the accuracy of method used.

5.1.2 Determination of Protein Concentration with Folin-Ciocalteu Reagent:

Principle: The method described below is that of Lowry et al. (1951). The final colour is result of, (i) biuret reaction of proteins with copper ions in alkali, and (ii) reduction of phosphomolybdic-phosphotungstic reagent by the tyrosine and tryptophan present in the treated protein.

Reagents:

Reagent A: 2 percent sodium carbonate (Na_2CO_3) in 0.1 N sodium hydroxide (NaOH).

Reagent B: 0.5 percent copper sulphate ($CuSO_4.5H_2O$) in 1 percent sodium or potassium tartarate.

Reagent C: Alkaline copper solution. 50 ml of reagent A was mixed with one ml of reagent B. It was discarded after one day.

Reagent D: Diluted Folin reagent. Folin Ciocalteu reagent (given below) was diluted by adding equal volumes of reagent and double distilled water.

Folin Ciocalteu Reagent (1927):

Mixture, consisting of 100 gm of sodium tungstate ($Na_2WoO_4.2H_2O$), 25 gm sodium molybdate ($Na_2MoO_4.2H_2O$), 700 ml of double distilled water, 50 ml of 85 percent phosphoric acid, and 100 ml concentrated hydrochloric acid, was refluxed gently for 10 hours. This was followed by addition of 150 gm of lithium sulphate, 50 ml of double distilled water, and few drops of bromine water. The above mixture was boiled without condenser for 15 minutes to remove excess bromine. After cooling it was diluted to one litre and filtered. Prepared reagent was stored in amber coloured bottle and away from light source. Standard curve for protein are shown in Figures 2 and 3 and were used to determine protein concentration in unknown sample, using following procedure:

To one ml solution consisting of 20 to 100 μgm of protein, 5 ml of reagent C was added. This was followed by vigorous mixing and was allowed to stand for 10 minutes. Finally 0.5 ml of reagent D was added with immediate mixing. After about 30 minutes, the absorbance was read against a suitable blank on spectrophotometer using a wavelength of 660 mμ and 1.5 cm light path.

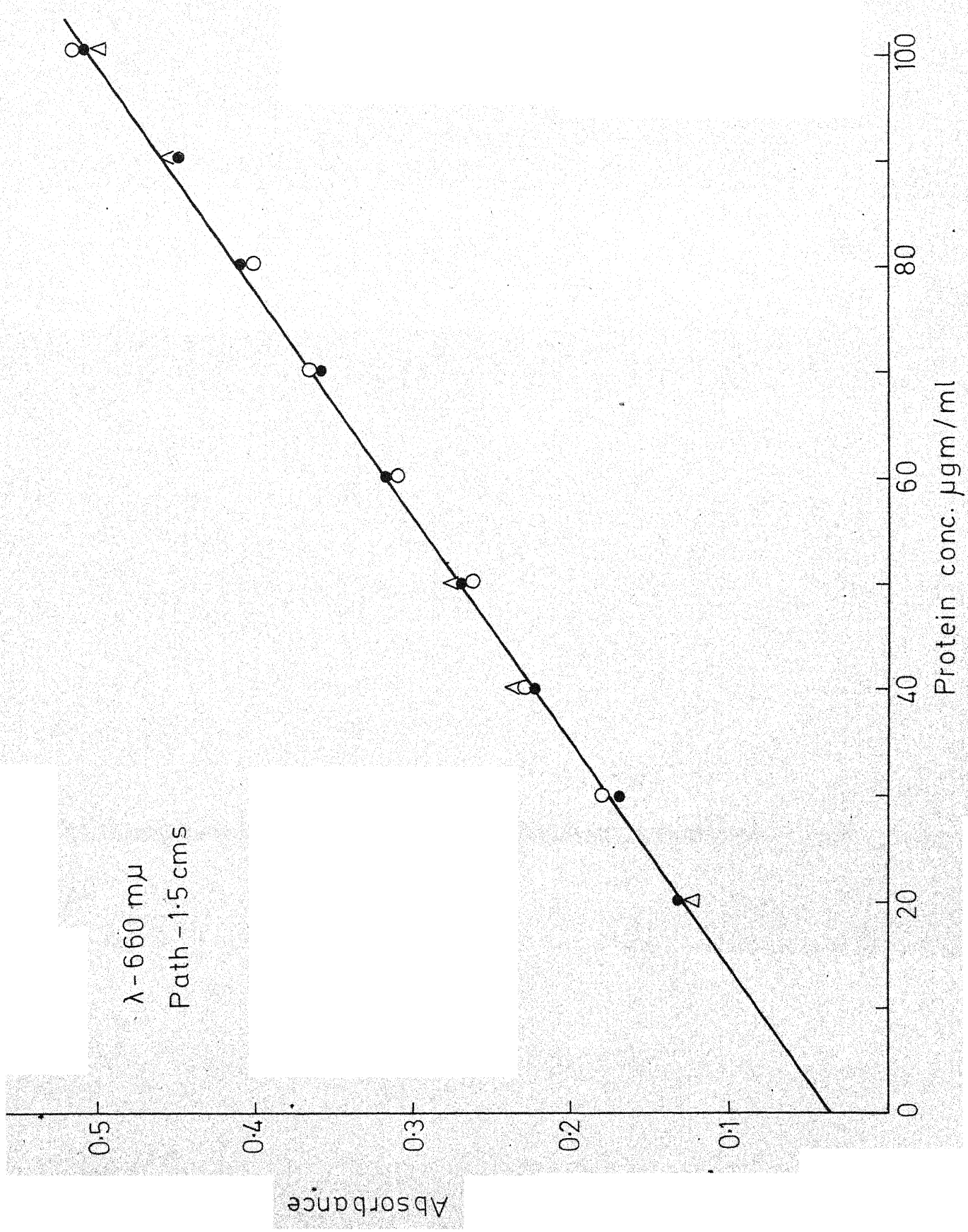


FIG. 2 PROTEIN STANDARD CURVE

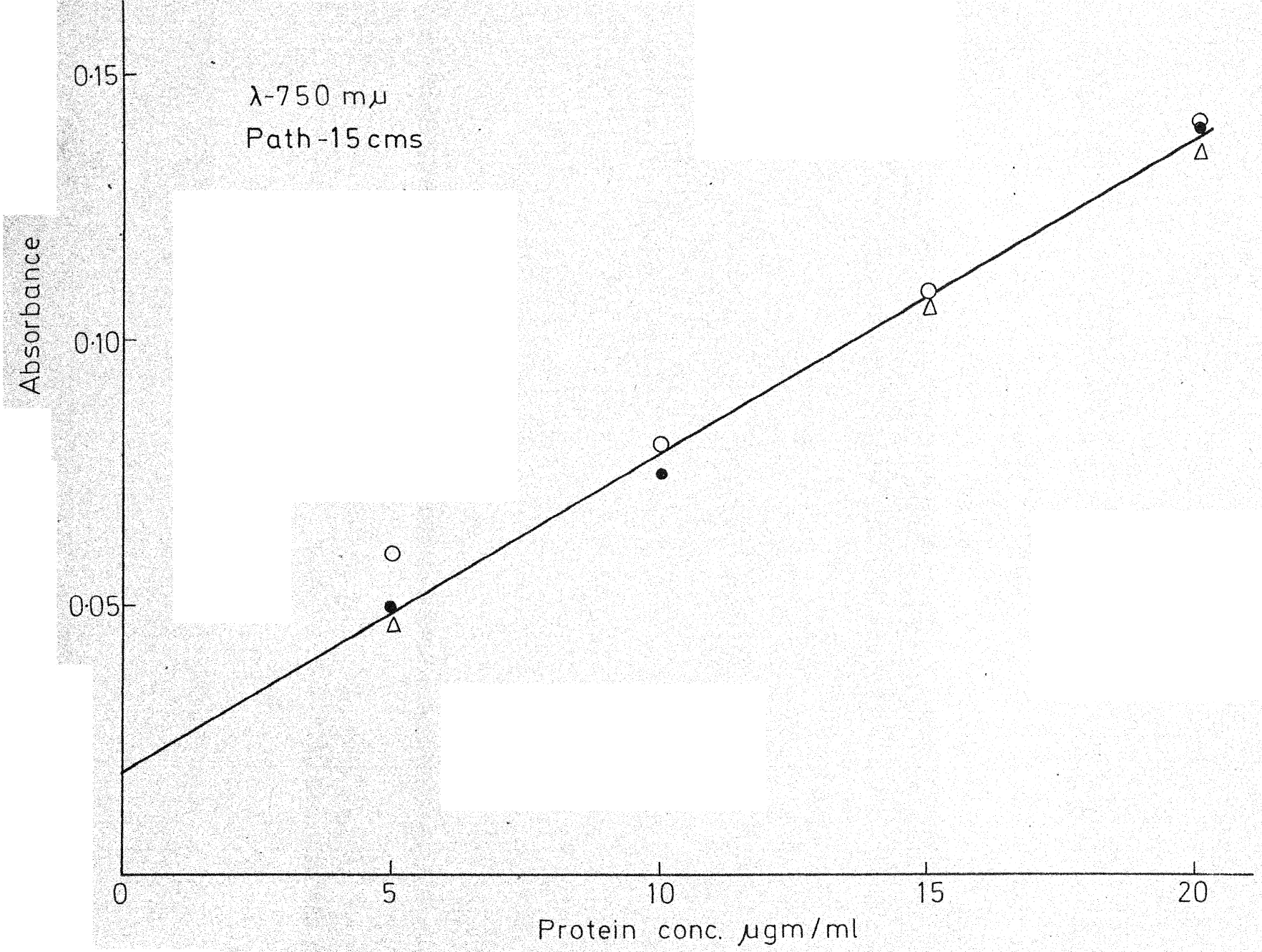


FIG. 3 STANDARD CURVE FOR PROTEIN AT LOW CONC.

If protein concentration was found to be less than 25 μgm per ml, one ml of protein solution was mixed with 1 ml of an exactly double strength reagent C, and otherwise was treated as mentioned above. For the range 5 to 25 μgm of protein per ml of final volume it was desirable to read absorbance using wavelength of 750 mμ.

Interference:

Neither colour nor interference with protein colour development has been observed with inorganic cations and anions. Lowry et al. (1951) observed no interference with protein colour development with Na_2SO_4 (1 percent), $NaNO_3$ (1 percent). Lowry et al. (1951) concluded that inorganics may affect colour development due to decrease in alkalinity caused by them.

5.2 Experimental Procedure

5.2.1 Glassware and Water:

All glassware used in Aluminium Hydrolysis, and Aluminium Protein interaction experiments, were cleaned by soaking them in concentrated sulphuric acid followed by rinsing in tap water. Afterwards the glassware were washed with detergent solution (Teepol, Surfactant Pvt. Ltd., Bombay) followed by rinsing in single and then in double distilled water. Glassware were dried in an oven.

Double distilled water was used in the experimentation. Conductivity of double distilled water, on an average, was 3 to 3.5 μsiemens/cm.

5.2.2 Study of Aluminium Hydrolysis

5.2.2.1 Kinetics of hydrolysis

Reaction mixture was obtained by adding 1 to 5 ml of 0.02 N KOH to a solution (200 ml) consisting 50 mg per litre of aluminium sulphate, and 0.01 M KNO_3. For proper mixing of KOH solution in the reaction mixture, magnetic stirrer was used. Just after addition of KOH, conductivity of the reaction mixture was measured. Conductivity measurements were carried out at the frequency of 30 seconds for initial one hour, and afterwards at the frequency of one hour for 8 hours. Temperature of reaction mixture was noted in the beginning as well as at the end of experiment.

Experiments were carried out in the manner explained above, but instead of conductivity measurements, pH measurements were carried out at the same time frequency mentioned earlier. For continuous pH measurements pH meter was coupled to a recorder (Digilog, Bombay). For greater accuracy in pH measurements following procedure was followed. Initially null adjustment was done, followed by direct measurement of solution potentials. Readings obtained were

in millivolts, which were converted to appropriate pH values. This was done with standard curve shown in Figure 4. Instrument calibration was done with number of buffer solutions (3.5 to 10.5) and noting the values of corresponding potentials in mV.

5.2.2.2 Equilibrium hydrolysis:

This study was conducted with six aluminium concentrations starting from $1.5x10^{-4}$ M to $9x10^{-4}$ M at the interval of $1.5x10^{-4}$ M concentration approximately. The concentration of an alkali (KOH) to be added was varied from 0.02 N to 0.12 N at the interval of 0.02 N. In all experiments ionic strength was maintained by KNO_3, and was equal to 0.01 M.

First 100 ml solution, containing 100 mg per litre of aluminium sulphate, was prepared. In the above solution 100 ml of alkaline solution was added with continuous stirring. The above mentioned alkaline solution was prepared by diluting 0 to 10 ml of KOH (0.02 N) to a final volume of 100 ml with double distilled water. When alkali solution was strong enough in comparison to aluminium concentration, the sequence of addition was reversed to avoid any heterogeneous distribution of hydroxyl ions, because of extreme concentration difference.

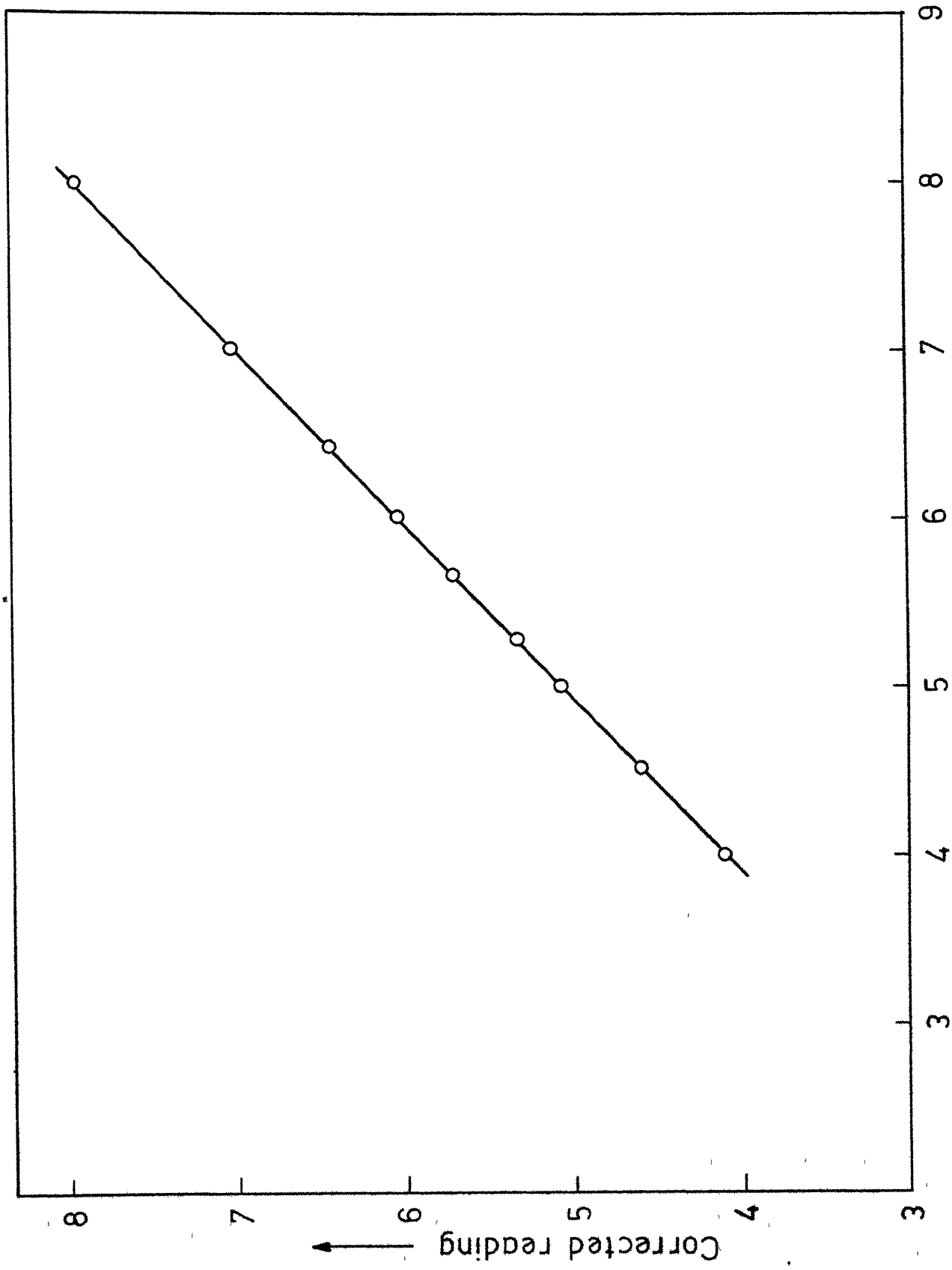


FIG. 4. pH METER CALIBRATION CURVE

Just after preparation of reaction mixtures turbidity measurements were carried out to see the extent of precipitation in each reaction mixture prepared. These measurements were repeated after 24 hours, also pH measurements were carried out at 2, 4, 12, 24, and 48 hours after preparation. From each reaction mixture 50 ml of supernatant was taken out, after about 24 hours, for quantitative estimation of aluminium, and OH^- bound to aluminium ions. Out of 50 ml sample 10 ml was added to 10 ml of sodium flouride (0.1 N) solution and remaining was titrated against 0.01 M HCl. The sample treated with sodium flouride was heated for 20 minutes, and after cooling its pH was measured. Appropriate blank was treated in the same manner. Difference in pH gives hydroxyl ion concentration bound to aluminium ions in the supernatant. The HCl (0.01 N) titration was performed with the help of pH meter. Titration end point was the pH of aluminium solution. This titration also gave concentration of hydroxyl ions bound to aluminium ions in the supernatant. The same sample was used for aluminium estimation.

5.2.2.3 Effect of SO_4^{--} ions on Al-hydrolysis

These experiments were carried out at three $(\frac{SO_4}{Al})$ molar ratios, and two aluminium sulphate concentrations (50, and 300 mg/l). The three molar ratios selected were

2, 2.5, and 3; sodium sulphate was used to provide excess sulphate ion concentration. The procedure of preparing reaction mixture was same as explained in section 5.2.2.2. Only pH measurements were carried out at the above mentioned time intervals.

5.2.2.4 Effect of medium maintaining ionic strength on hydrolysis of aluminium:

In all the above experiments KNO_3 was used in maintaining ionic strength at 0.01 M. Instead of KNO_3, KCl was used of same concentration i.e. 0.01 M. Experiments were carried out in similar fashion as explained in section 5.2.2.2.

5.2.3 Al-Protein Interaction:

5.2.3.1 Kinetics of Al-protein reaction:

Borine serum albumin was the protein used in the experiment. Stock solution was prepared of 1 gram per litre strength, 20 ml of stock solution was diluted ten times to obtain 200 ml solution with 100 mg per litre of protein concentration. This solution was transferred to a beaker, and beaker was kept on a magnetic stirrer. This was followed by addition of one ml of aluminium sulphate to give final concentration of 50 mg per litre of aluminium

sulphate in the solution. Mixing continued till the end of experiment. At 15 minutes frequency, samples were drawn for protein estimation. The samples drawn were first centrifuged at 600 rpm for 15 minutes and the supernatant was used for protein estimation.

One alternative method was used. Proteins have a distinct absorption maxima in ultraviolet range. As reaction between proteins and aluminium would continue, soluble protein concentration would start diminishing. Reaction mixture was prepared in the same manner and was transferred to a absorption cell. Against a suitable blank absorption was measured at 280 mμ wavelength. For this purpose the DU-2 Spectrophotometer (Model DU_2, Beckman I Inc., U.S.A.) was used.

5.2.3.2 Protein titrations:

Potentiometric titrations were carried out for protein solutions. Protein solution containing 100 mg per litre of protein was titrated against 0.1 N HCl and 0.1 N NaOH. This was done in order to understand dissociation as well as uptake of hydrogen ions by protein molecules at different pH values. Titrations were also carried out for the blank without proteins.

To avoid error in pH measurements caused by adsorption of protein molecules on the membrane of glass electrode, glass electrode was dipped in 0.1 N HCl after each 15 minutes of use.

5.2.3.3 Al-protein interaction at different pH values:

In the present investigation no buffer system was used in maintaining any pH value of reaction mixture. Buffer system, if provided, may affect in the following manner.

(1) Ionic strength of the solution is increased than what is decided i.e. 0.01 M.

(2) Protein interaction with buffer will unnecessarily complicate the system.

(3) Al interaction with buffers like, PO_4 buffer may complicate the system to be studied which by itself is sufficiently complicated.

Instead of studying the interaction at specified pH, it was decided to study at specified $(\frac{KOH}{Al})$ molar ratios. Aluminium concentrations used were the same as that used in hydrolysis experiments. Following $(\frac{KOH}{Al})$ molar ratios were selected: 0, 1, 2, 3, and 4, which would cover the necessary pH range from 4 to 10. Protein concentrations used were from 50 mg per litre to 200 mg per litre.

Initially 200 ml of protein solution was prepared with 50-200 mg per litre of protein. This was followed by an addition of 1 ml of aluminium sulphate and one ml of KOH, to give 50-300 mg per litre of aluminium sulphate concentration and appropriate $(\frac{KOH}{Al})$ molar ratio. Estimation procedure being slightly different for solutions with $(\frac{KOH}{Al})$ molar ratio = 0, when compared to other, molar ratios, they are given separately:

(1) $(\frac{KOH}{Al}) = 0$:

As aluminium is only in the form of ions, the system becomes somewhat easy to deal with. After 24 hours, 50 ml sample was taken out from reaction mixture. This sample was centrifuged at 6000 rpm for 15 minutes. Protein concentration in the supernatant was determined by the procedure given earlier. Determination of Al by the suggested method showed wide variations. Therefore alkalimetric titrations (Hahn, Stumm, 1968) were used for aluminium determination. But this method is useful for aluminium concentration greater than 10^{-4} M. This method is as accurate as the colourimetric method employed. Out of 50 ml centrifuged sample, 40 ml of supernatant was added to 10 ml of 0.02-0.12 N KOH. After half an hour, it was titrated against 0.02-0.12 N HCl. Titrations were performed with

the help of pH meter. Titration curve show two inflexion points; first one due to neutralization of OH^- ions and the other one due to transformation of $Al(OH)_4^-$ to lower hydroxyl species. From this, amount of OH^- ion reacted with aluminium ions can be calculated and hence aluminium ion concentration determined.

(2) $(\frac{KOH}{Al}) > 0$:

With addition of alkali complexities are introduced as one has to deal with two reactions simultaneously. These two reactions are (i) Al-protein reaction, and (ii) Al-hydrolysis.

Depending on Al-protein reaction, equilibrium hydrogen ion concentration will show a shift when compared to hydrolysis reaction at the same $(\frac{KOH}{Al})$ molar ratio but in absence of protein. This change in equilibrium pH will show the amount of Al reacted with protein. Therefore, after centrifuging, pH measurements of supernatant as well as protein estimation was done.

5.2.3.4 Effect of Ca and Mg on Al-protein interaction:

Ca and Mg are known to form complexes with proteins and hence can be assumed to compete with aluminium ions, for the sites existing on protein molecule. Both these

cations form bidentate chelates with carboxylic groups, e.g. Ca-Edta complex, Mg-Edta complex. Their concentration in wastewater is also high (150-250 mg per litre) while studying their effect on Al-protein interaction, two concentration were chosen and those are 50, 100 mg per litre.

Experiments were carried out in similar fashion except for addition of Ca and Mg. Equilibrium pH, and protein concentration were determined.

5.2.3.5 Effect of diprotic organic acids on Al-protein interaction:

Aluminium is known to form complexes with fatty acids, causing increase in soluble aluminium concentration in solution (Sillen and Martell, 1972). These acids may compete with proteins, depending upon their concentration as protein molecules also consist of variable number of -COOH groups. Not only from theoretical point of view, but this study is also for practical reasons. Since early degradation of organic matter in wastewater leads to formation of lower fatty acids or volatile acids. If aluminium compounds are used in treatment plants for chemical precipitation the effect of these acids, on the aggregation of macromolecules like proteins will be of considerable importance.

Mainly two acids were studied, (i) oxalic acid, and (ii) malic acid. The experimentation was carried out in a similar way as explained earlier, with the addition of these two acids. Final concentration in reaction mixture was adjusted to 40 mg per litre. Equilibrium pH and supernatant protein concentration were determined.

CHAPTER - VI

RESULTS AND DISCUSSION

6.1 Aluminium-Sulphate Hydrolysis:

6.1.1 Kinetics of hydrolysis:

Experimental results of the reaction kinetics are shown in Figure 5. It is evident from the results, that the reduction in conductivity is only limited to initial 5 minutes, as it remains constant afterwards. Hydrolysis of metal ions can be assumed to be a two step reaction,

(i) formation of monomers followed by

(ii) formation of polynuclear complexes

The aluminium ion concentration used in the experiment being quite low 1.57×10^{-4} M , conductivity variations can be assumed to be directly proportional to the disappearance of aluminium ions from the solution. Therefore the rate of reduction in conductivity will be directly proportional to the rate of monomer formations, followed by further reduction in conductivity with the formation of polynuclear complexes as conductivity is a function of charge and size of the ions. The results of the reaction kinetics show no variations in the conductivity after initial period of 5 minutes approximately. Therefore it can be inferred that the reaction is

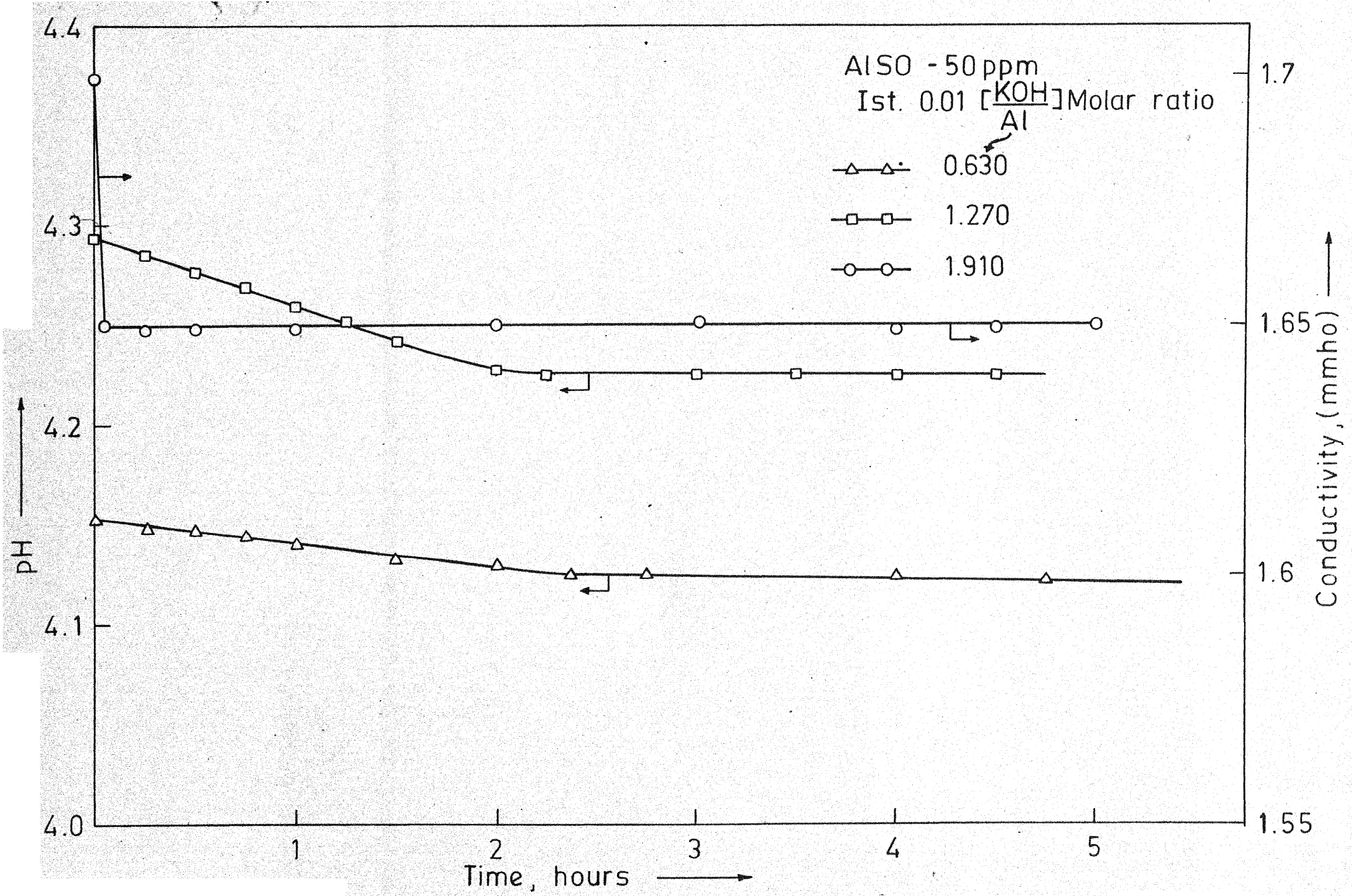


FIG.5. KINETICS OF HYDROLYSIS

fast, and even formation of polynuclear species is complete within the initial period of 5 minutes.

In the same Figure 5, variation in pH with time has been shown. It can be observed that the reduction in pH is directly proportional to time, for initial two hours, and afterwards gradually reaches equilibrium. Reduction in the pH indicates that reaction is not complete in the initial 5 minutes as concluded from the conductivity measurements. The present system can be represented by following equation,

Rate of removal of OH^- =

Rate of monomer formation +

Rate of reaction between OH^- and polynuclear complexes

(32)

i.e.

$$-\frac{dC_{OH^-}}{dt} = k_1\left[C_{Al^{3+}}\right]^x\left[C_{OH^-}\right]^y + k_2\left[C_{OH^-}\right]^l\left[C_P\right]^m \quad (33)$$

where k_1, k_2 - rate constants

x, y, l, m - constants

C_{Al^+} - concentration of Al^{3+} at time 't'

C_{OH^-} - concentration of OH^- at time 't'

C_P - concentration of polynuclear composition at time 't'.

In metal complex formation reactions, a water molecule in the co-ordination sphere of a cation is replaced by a ligand. Since such water molecules are tightly bound, their substitution may be considerably slower than the rate of diffusion and may represent the rate determining step in the overall process. But when concentration of metal ions is considerably higher than that of ligand the rate of diffusion will be deciding parameter of the overall reaction rate. In the present case $C_{Al^{3+}} \gg C_{OH^-}$, and therefore equation (33) can be written as,

$$-\frac{dC_{OH^-}}{dt} = k_1'\left[C_{OH^-}\right]^y + k_2'\left[C_{OH^-}\right]^1 \qquad (34)$$

where $k_1' = k_1\left[C_{Al^{3+}}\right]^x$

$k_2' = k_2\left[C_P\right]^m$

As concentration of OH^- in the solution is low enough, the rate of diffusion of OH^- ions can be assumed to be directly proportional to C_{OH^-}, therefore putting $y = 1 = 1$

$$-\frac{dC_{OH^-}}{dt} = \bar{K} \cdot C_{OH^-} \qquad (35)$$

where $\bar{K} = k_1' + k_2'$

Integrating equation (35) we get,

$$- \ln(OH^-) = \bar{k} \cdot t + C_1 \qquad (36)$$

where C_1 - integration constant.

at $t = 0, \quad C_{OH^-} = \bar{C}_{OH^-}$

therefore equation (36) can be written as

$$- \ln C_{CH^-} = \bar{k} \cdot t - \ln \bar{C}_{OH^-} \qquad (37)$$

$$+ \ln \frac{\bar{C}_{OH^-}}{C_{OH^-}} = \bar{k} \cdot t \qquad (38)$$

Equation (38) can be expressed as,

$$pH(t) - pH(t = 0) = -\frac{\bar{k}}{2.3} \cdot t \qquad (39)$$

as $\ln \frac{1}{C_{OH^-}} = - pH(t) \cdot \left[\frac{1}{2.3}\right]^{-1}$ = hydrogen ion conc. at time 't'.

Equation (39) represents the kinetics of the hydrolysis reaction, for initial 1 hour and 30 minutes.

Brosset (1952), has observed that the hydrolysis of aluminium ions was a second order reaction when $\bar{n} < 1.5$, and a first order reaction when $3 < \bar{n} < 4$. The equations proposed by him representing the kinetics of hydrolysis reaction were,

$$\frac{1}{C_{OH^-} - C_{OH^-}(\infty)} = k't + C_1 , \qquad (40a)$$

when $\bar{n} < 1.5$

and $$\log \left[C_{OH^-} - C_{OH^-}(\infty) \right] = k''t + C_1' , \qquad (40b)$$

when $3 < \bar{n} < 4$

where C_1 and C_1' - integration constant

$C_{OH^-}(\infty)$ - equilibrium concentration of OH^-

C_{OH^-} - concentration of OH^- at time 't'.

The results shown in Figure 5 are for $\bar{n} < 1.5$, and equation (39) represents the kinetics of aluminium hydrolysis. The equation proposed by Brosset (1952) is different from equation (39). The equation (39) is not derived from well defined reaction mechanism, and therefore is not applicable after about 2 hours when the system gradually attains equilibrium. This may be the reason that equation (39) differs from the equation proposed by Brosset (1952).

6.1.2 Study of hydrolysis equilibrium:

Study of aluminium hydrolysis equilibrium was performed using six different aluminium ion concentrations, concentration used were 1.5×10^{-4} M to 9.0×10^{-4} M approximately. Equilibrium diagrams are presented in Figures 8 to 13.

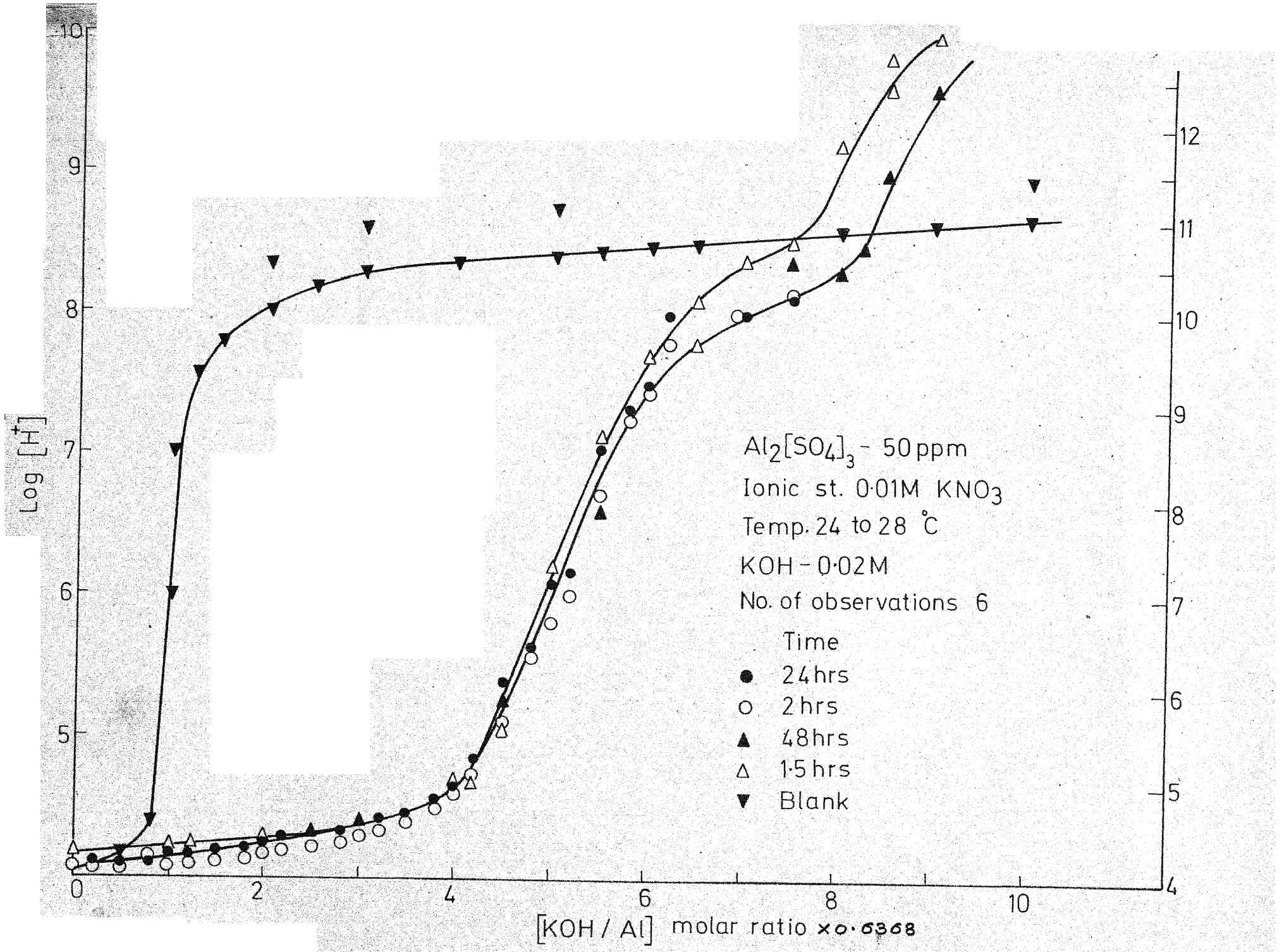


FIG 8 ALUMINIUM HYDROLYSIS

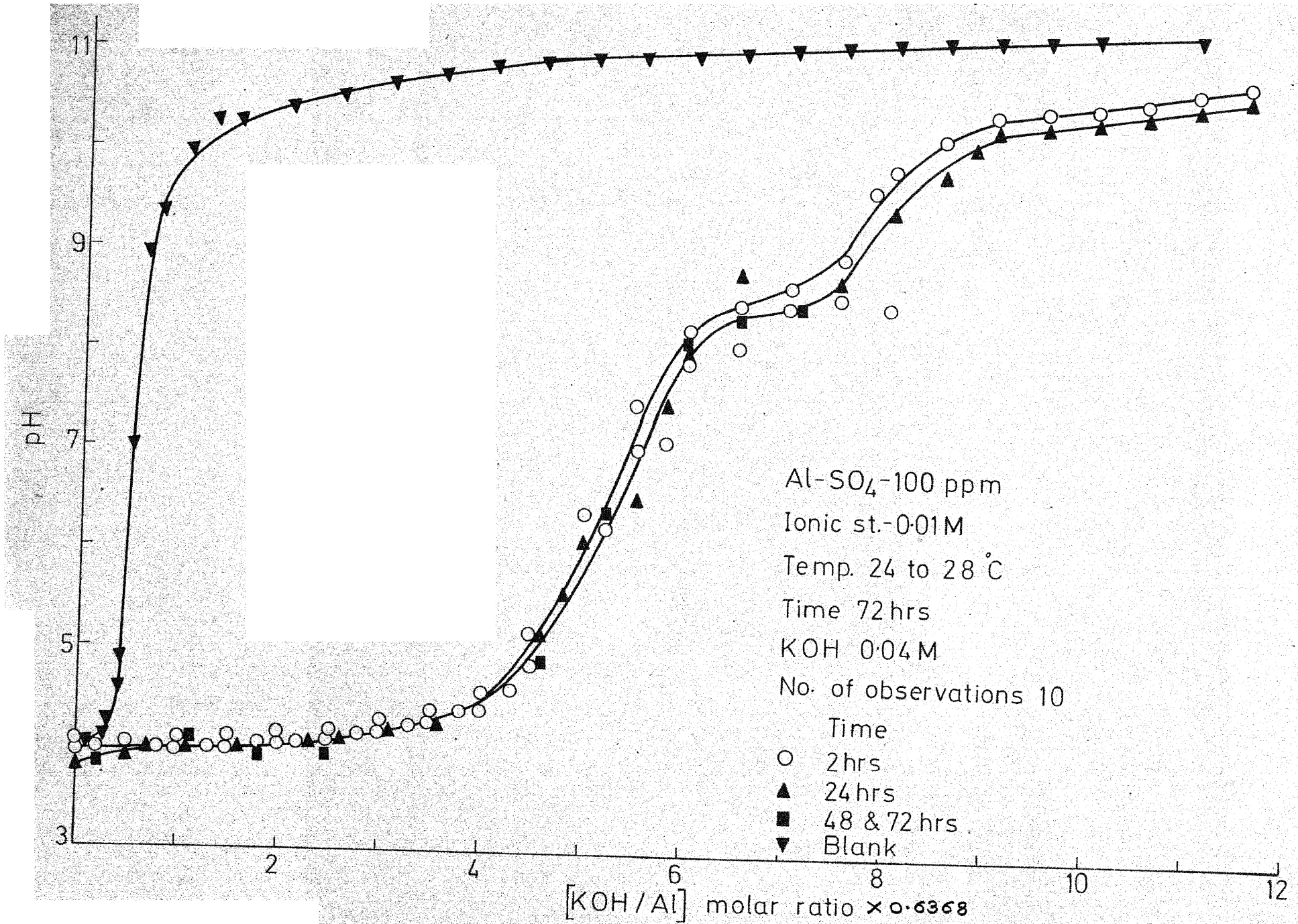


FIG. 9 ALUMINIUM HYDROLYSIS

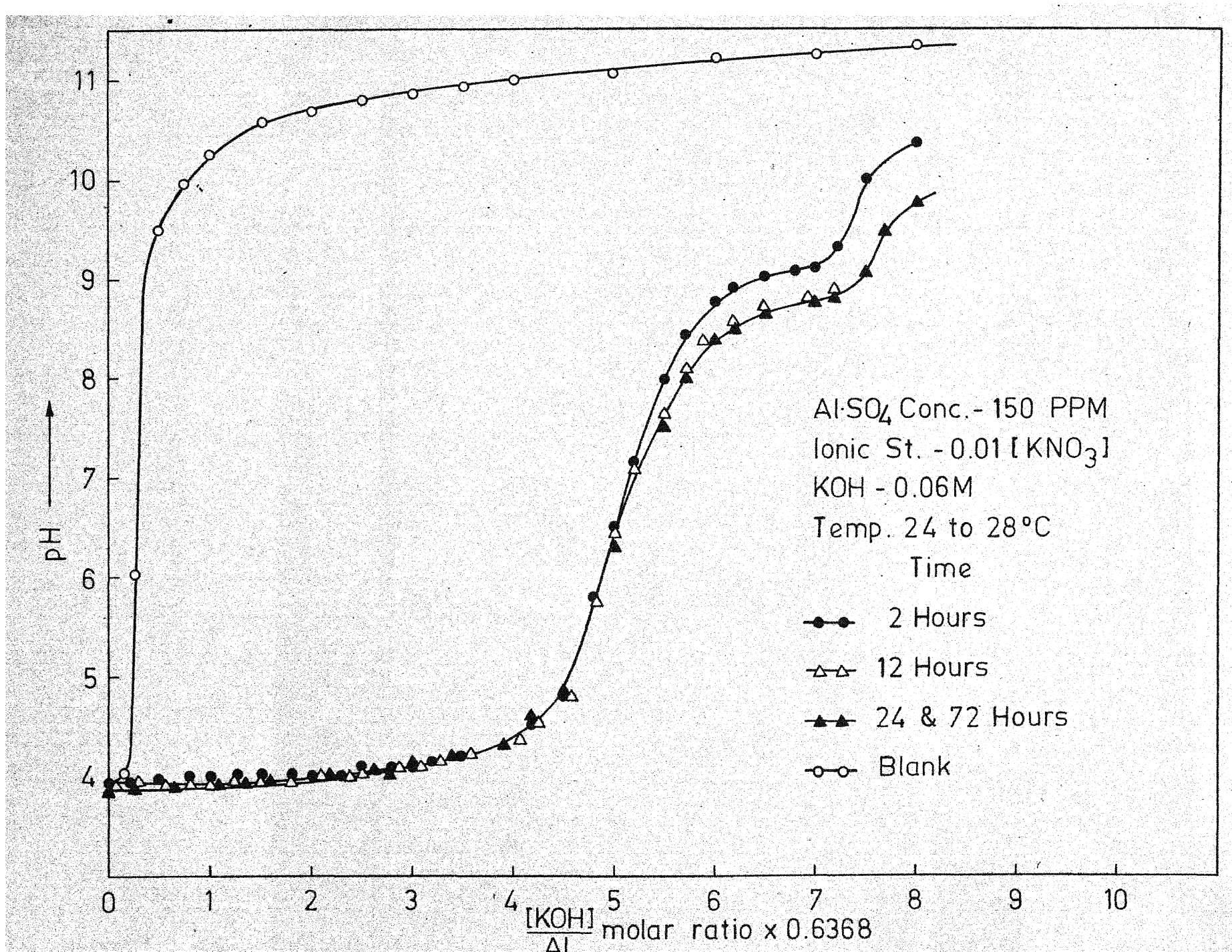


FIG. 10. ALUMINIUM HYDROLYSIS

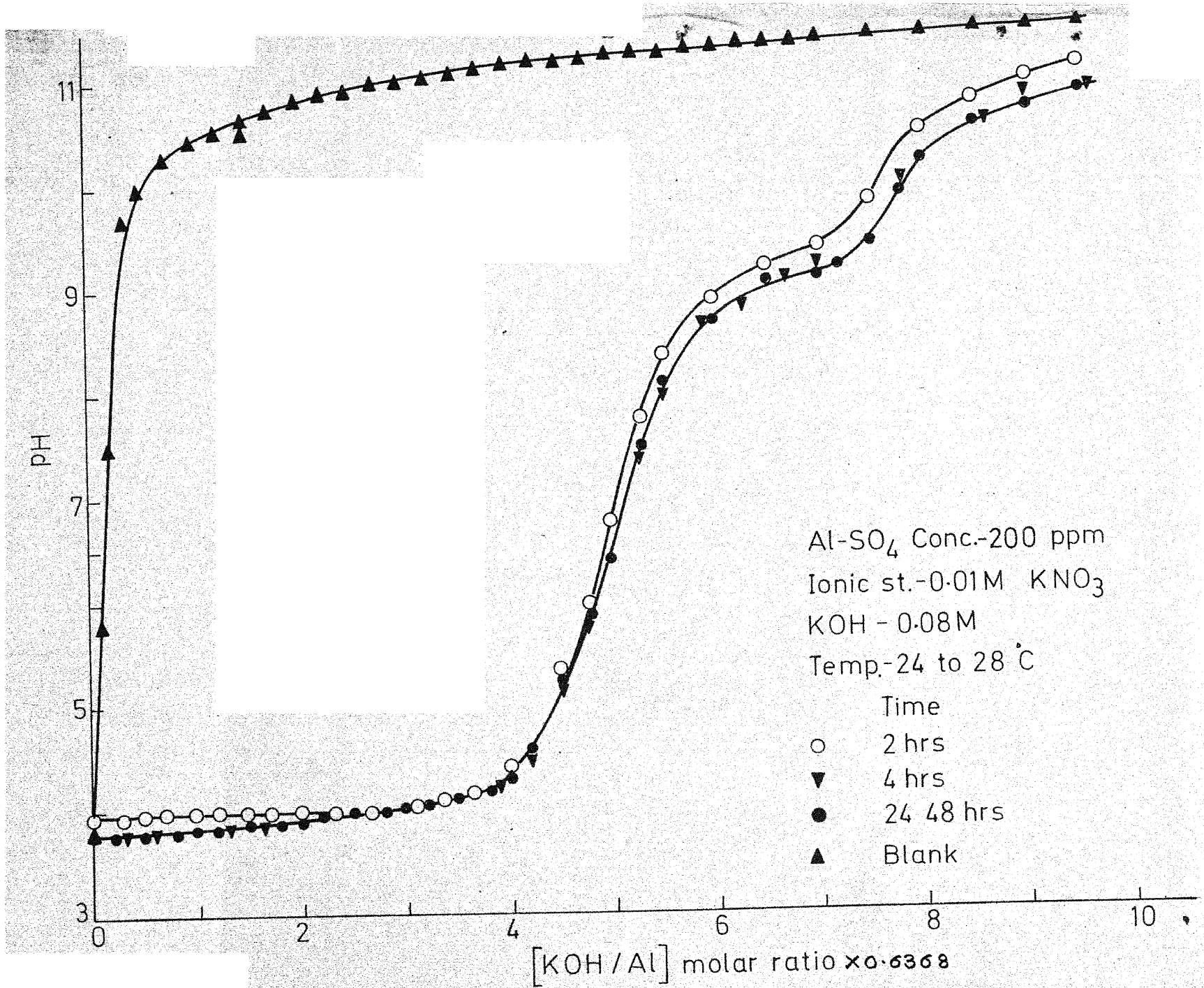


FIG.11 ALUMINIUM HYDROLYSIS

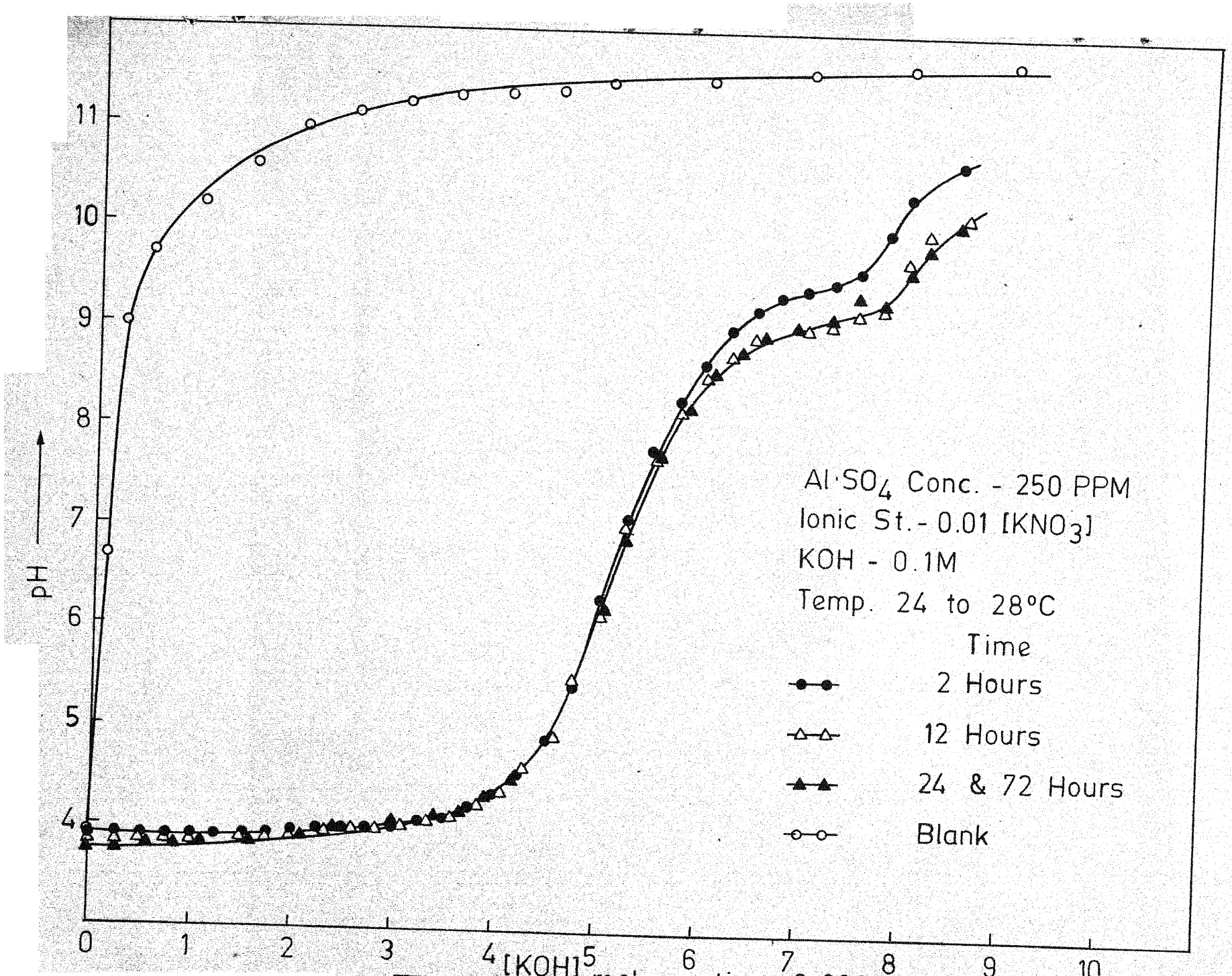


FIG.12. ALUMINIUM HYDROLYSIS

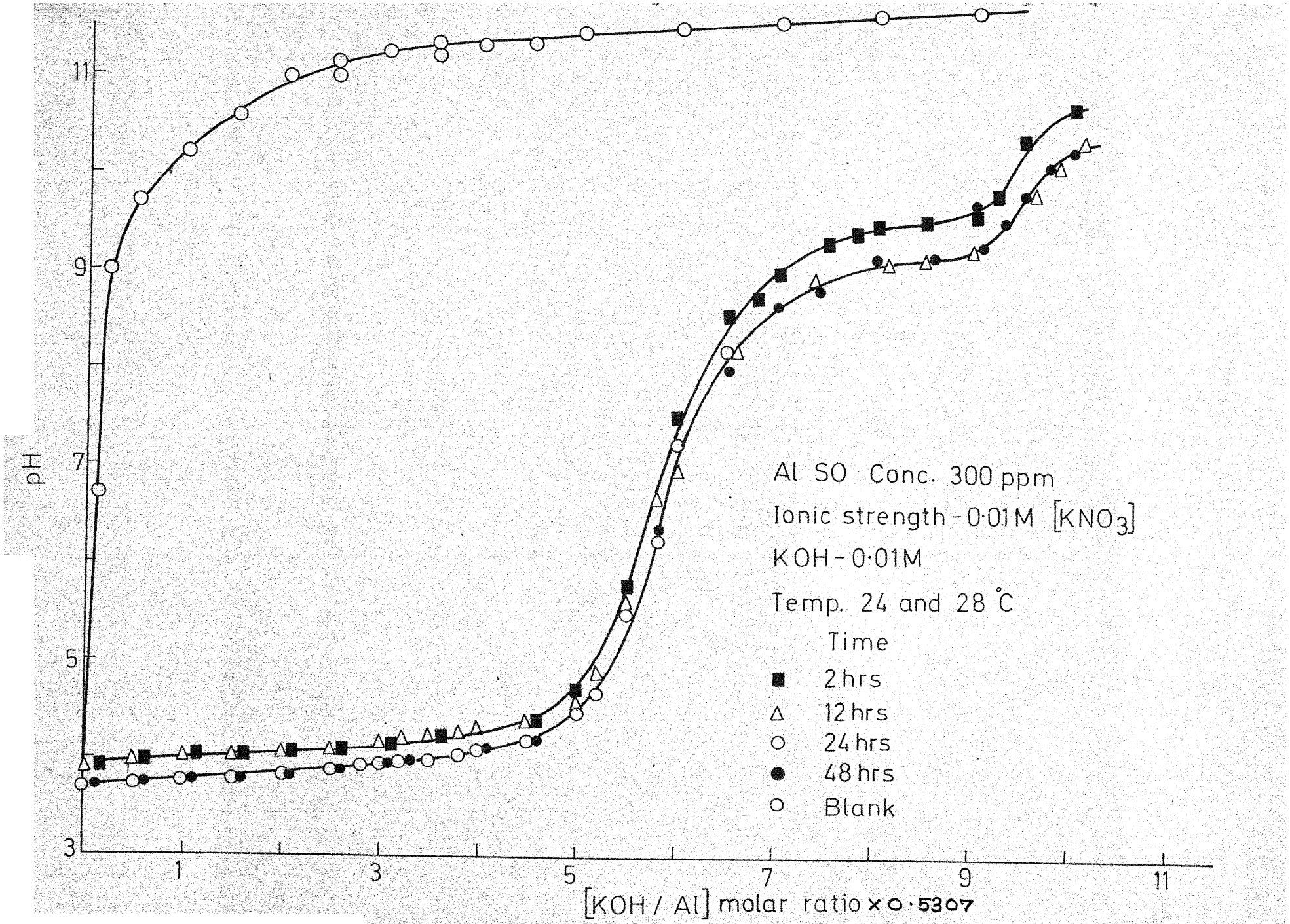


FIG. 13 ALUMINIUM HYDROLYSIS

To know the extent of precipitation, turbidity measurements were carried out, and are shown in Figure 6,7. The extent of precipitation seems to be negligible with aluminium concentration equal to 1.5×10^{-4}. Visual observations showed almost no precipitation. But in case of all other aluminium concentration it can be seen that precipitation is almost complete upto pH 4.35. If one plots the concentration of aluminium and the pH 4.35, the point lies considerably outside the $Al(OH)_3$ zone in the equilibrium plot, and discards the probability of precipitate having $Al(OH)_3$ as its composition. From the material balance of aluminium, and OH^- ion in supernatant the average composition of precipitate was calculated with the assumption that no other cations participate in the formation of precipitate. The average composition of precipitate is $Al(OH)_m(SO_4)_n$ where m = 2.4 and n = 0.3. Hsu, and Bates (1964) have worked with aluminium sulphate hydrolysis, the concentration chosen in their investigation was considerably higher in comparison to concentration used in present investigation. According to them the aluminium precipitate had the composition $Al(OH)_{2.2}(SO_4)_{0.4}$ which is slightly different from the present investigation. This may be because of low concentration of aluminium used. Tendency of metal ion solutions to protolyze or hydrolise increases with dilution. Perhaps this may be the reason for getting different constitution to that from Hsu, and Bates (1964).

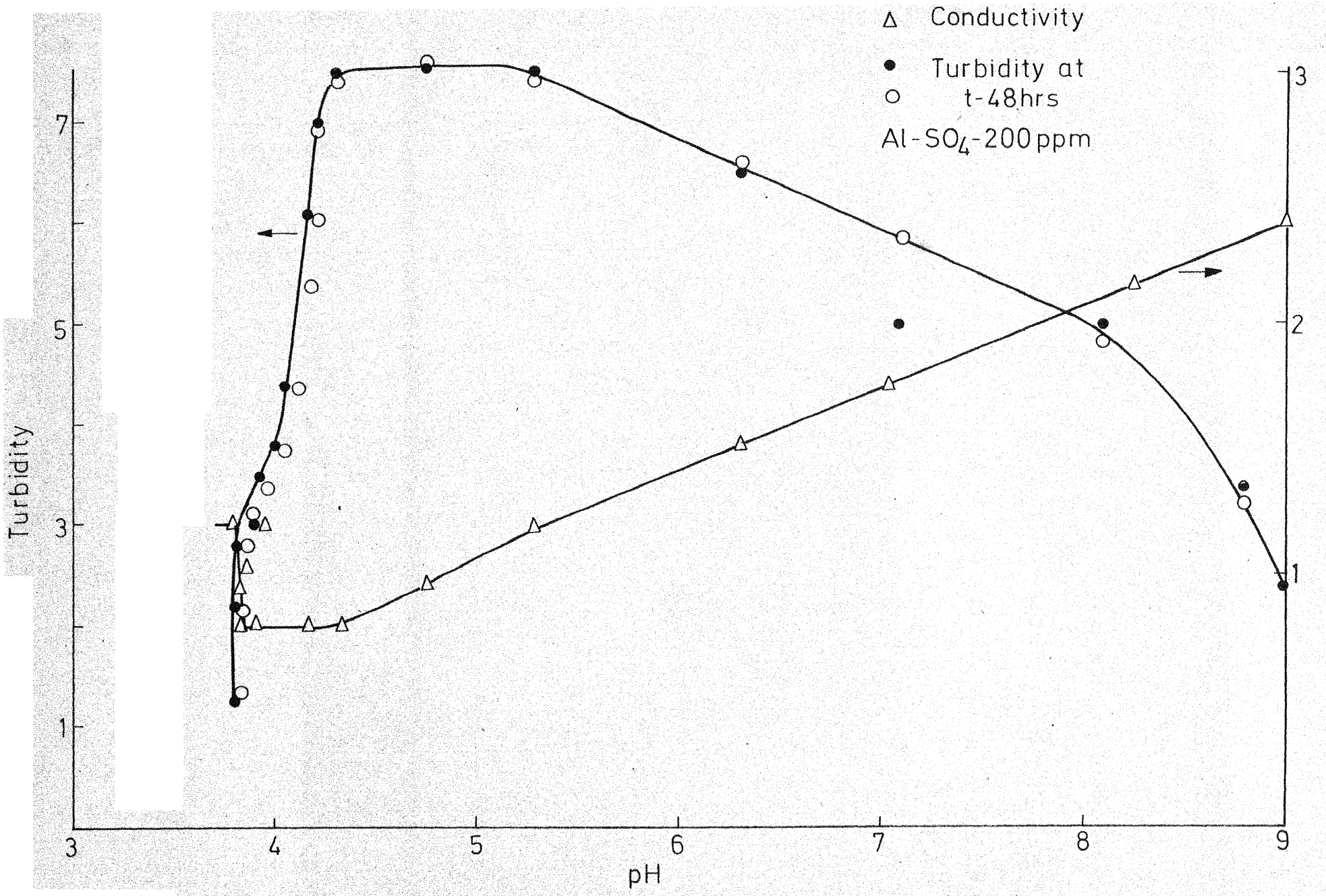


FIG. 6 TURBIDITY & CONDUCTIVITY VARIATIONS WITH H^+ ION CONC.

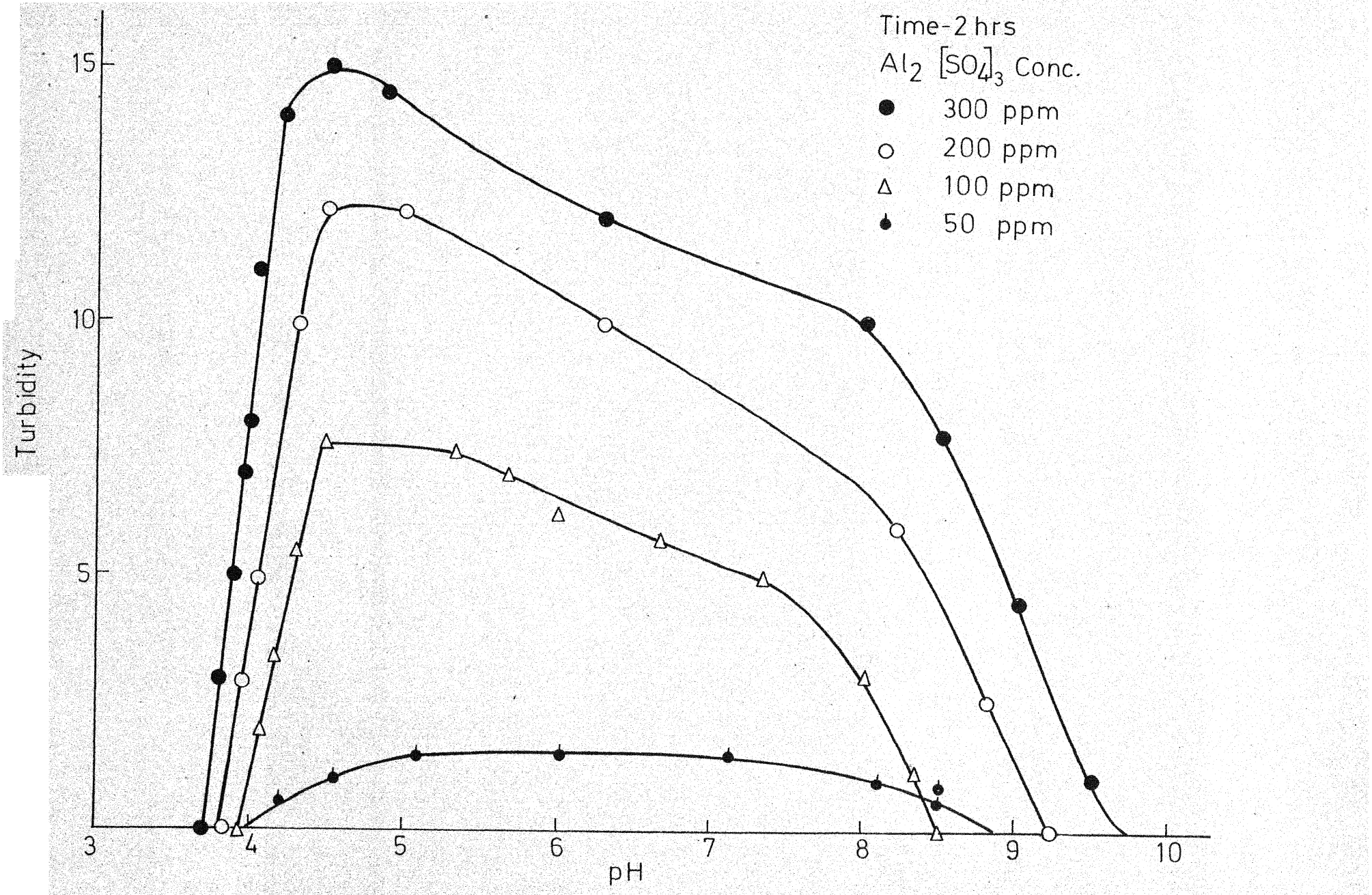


FIG. 7 TURBIDITY VARIATION WITH HYDROGEN ION CONC.

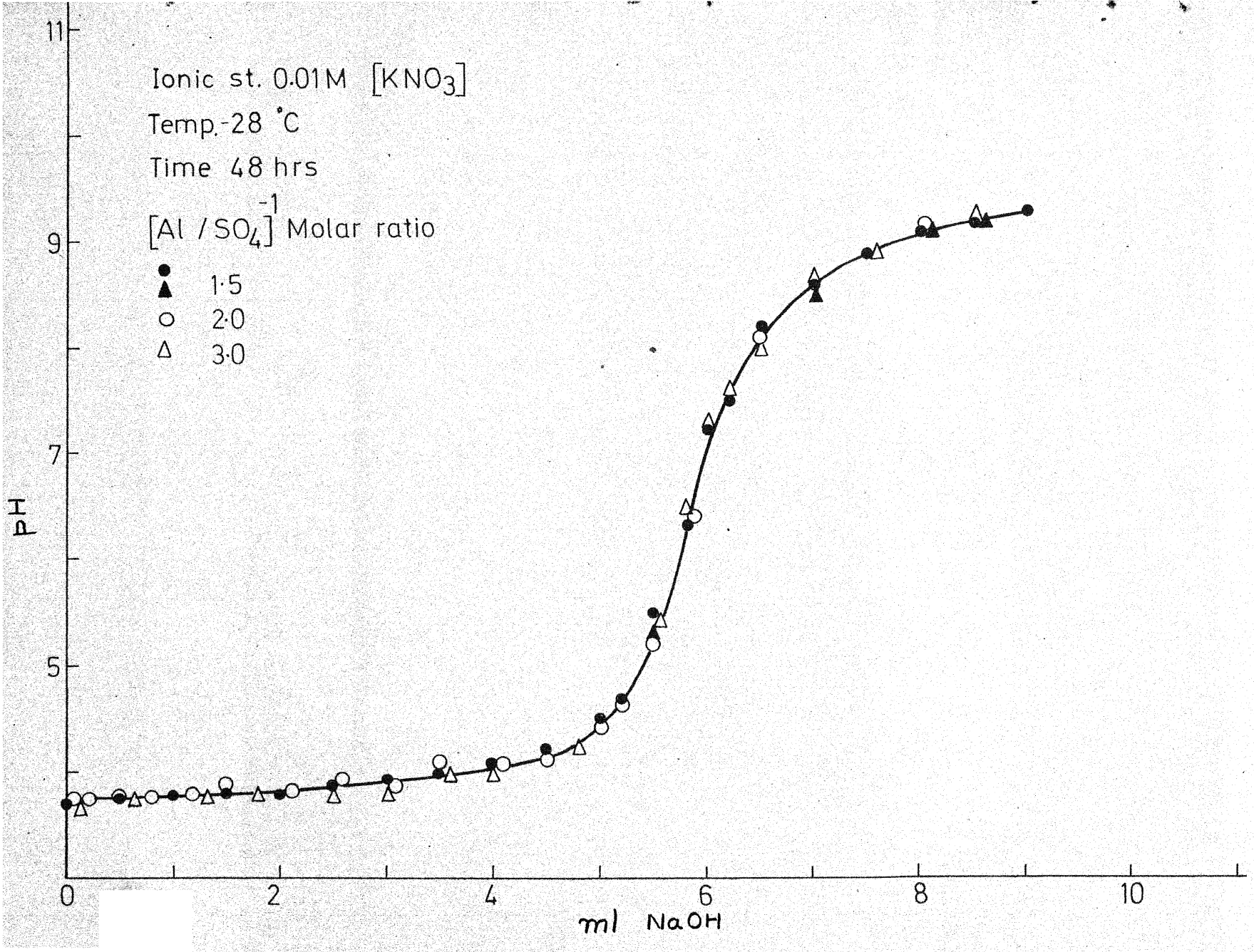
Sulphate ions seems to bridge up the polynuclear species, to form precipitate at early stages. The first inflexion point in the equilibrium curves show completion of precipitate formation with $Al(OH)_{2.4}(SO_4)_{0.3}$ as its composition. The precipitate formed were found to dissolve in sulphuric acid (IN) in a very short time. Showing probably no crystalline structure even after four days. Weiser, and Milligan (2939) have found that the precipitate formed from aluminium sulphate solution is X-ray amorphous. This is substantiated by Hsu, and Bates (1964) as well as McHardy and Thomson (1971). This shows that the precipitate is more heterogeneous in its character.

The precipitate composition linearly varies with pH. Very low $(\frac{Al}{OH})^{-1}$ molar ratio in supernatant indicates the absence of polymeric species in the supernatant. This can be explained in following manner. With the addition of alkali in aluminium sulphate solution, monomeric species are formed. These species undergo condensation reactions to form basic hexagonal unit, these hexagonal units are joined by sulphate ion bridges. As sulphate ion concentration is higher to that of aluminium most of the primary hexagonal units will be joined by SO_4 ion bridges and thus precipitate. Polynuclear species concentration depends on the remaining primary hexagonal units in the solution. As most of these units have precipitated leaves almost none of them in the solution. Therefore predominant species in the supernatant are all monomeric.

Stability constants:

Conductivity and solubility measurements show that the most SO_4^{--} are very highly associated with metal ions even in dilute solutions (Robinson, and Stokes (1959); Davies (1959)). The Raman frequencies (Hester, and Plane (1964)) suggest that, with single exception of In, the metal sulphate complexes are solvent separated at least one molecule of water trapped between metal ion and SO_4^{--}. Therefore from above observations it can be concluded that SO_4^{--} ions form outer sphere complexes with aluminium ion. Matwiyoff et al. (1968) have found the presence of outer sphere complex in the presence of NO_3^- with aluminium ions in the solution. Hsu, and Bates (1964) observed no change in equilibrium pH even at variable $(\frac{SO_4}{Al})$ molar ratio, which is also observed in the present investigation. (Fig. 14, and 15) Therefore it can be assumed that, SO_4^{--} ions do not interfere in the hydrolysis reaction and its equilibrium to the effect to consider them in the reaction under consideration.

The $\bar{n}(pH)_{B=c}$ curves are shown in Figure 16 for a series of Al(III) concentrations. The curve for lowest Al(III) concentration used, i.e. 1.57×10^{-4} M, exhibit a different trend when compared to rest of the curves shown in Figure 16, hence it has been discussed separately, but in the light of results obtained from the rest of the curves. The $\bar{n}(pH)_{B=c}$ curves can be divided into two distinct regions.



FIG·14 EFFECT OF SO_4 IONS ON ALUMINIUM HYDROLYSIS

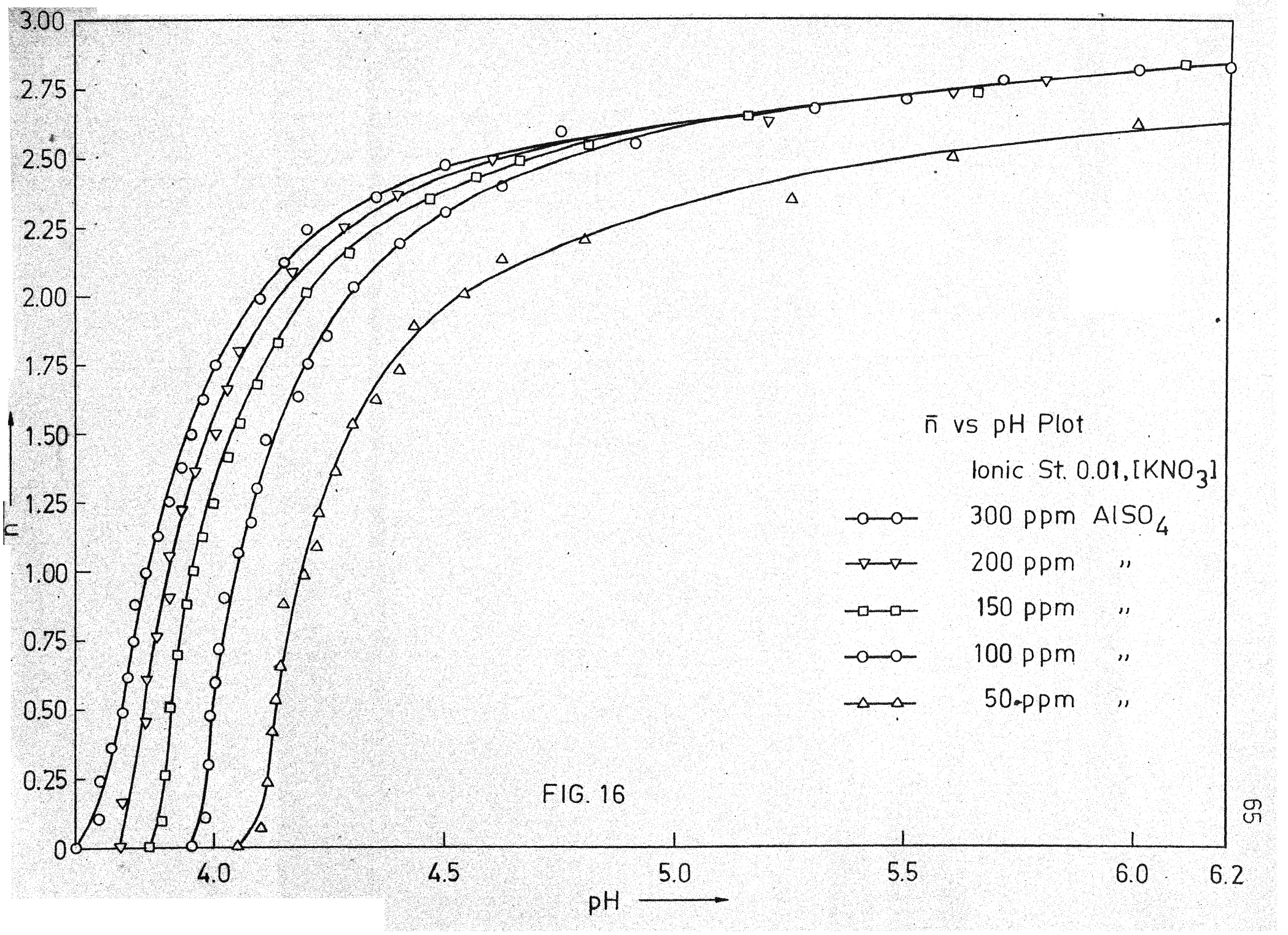

3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0
n̄
4.0
4.5
5.0
5.5
6.0
6.2
pH
n̄ vs pH Plot
Ionic St. 0.01, [KNO3]
300 ppm AlSO4
200 ppm ,,
150 ppm ,,
100 ppm ,,
50 ppm ,,

FIG. 16

(i) pH $<$ 5.1, where $0 < \bar{n} < 2.6$,

and (ii) $5.1 \leq$ pH ≤ 7.0, where $2.6 \leq \bar{n} \leq 3$.

In the region (i) curves are parallel to each other and gradually coincide at pH 5.1, and $\bar{n} = 2.6$, whereas region (ii) show that the $\bar{n}(pH)_{B=c}$ are coincident. Therefore in the region (ii), $\bar{n}$ is independent of Al concentration, and thus indicates presence of homonuclear species. Initially theoretical analysis of region (i) is carried out followed by region (ii). Systems of polynuclear complexes which gives rise to set of parallel curves, $\bar{n}(pH)_B$, have been treated by Sillen (1954), and Rossotti, and Rossotti (1956). Such systems were analysed in terms of a series of polynuclear complexes formed between a central group, denoted the core, and hypothetical ligand which also contain central group, referred to as links.

In the region $0 < \bar{n} < 2.6$, and pH $<$ 5.1, the function $\bar{n}(pH)_B$ are each parallel but not equidistant, which is evident from Figure 17 showing log $B(pH)_{\bar{n}=c}$ curves. The curvature at lower values of 'B' show presence of mononuclear species in the solution. The log $B(pH)_{\bar{n}=c}$, Figure 17, curves are almost parallel to each other in the range $0.5 \leq \bar{n} \leq 2.5$. Presence of mononuclear species at $\bar{n}$ values as high as 2 to 2.5, resulting in increased curvature at lower values of B, seems to be improbable. Therefore it can be assumed in the

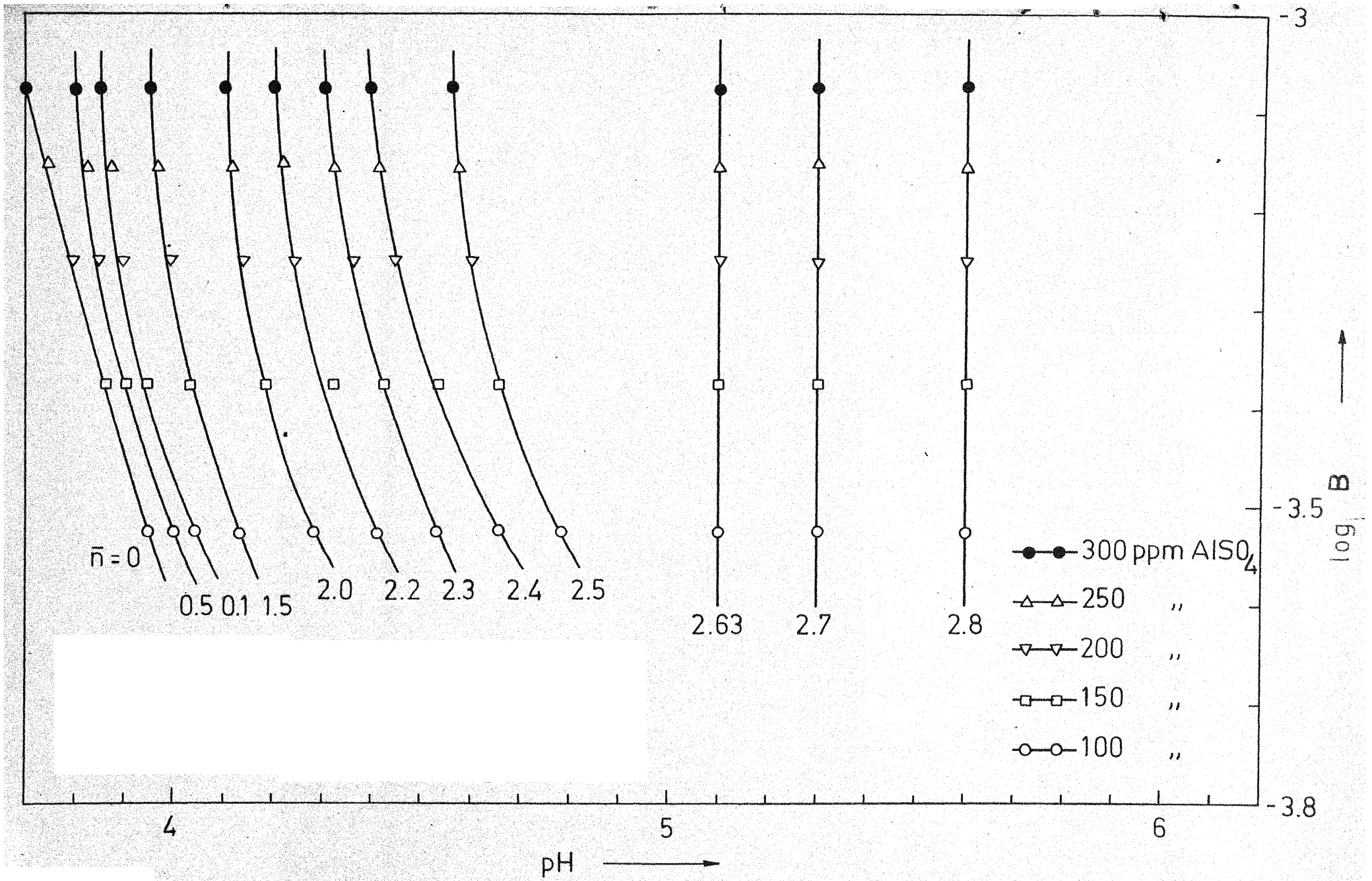


FIG. 17. pH vs log B CURVES AT CONSTANT n̄

present case that log $B(pH)_{\bar{n}=c}$ curves are set of parallel lines, thus indicating presence of unique complex in the solution.

The approximate spacing

$$t = -\left(\frac{\log B}{\log h^{-1}}\right) \qquad (41)$$

of the set of parallel curves $\bar{n}(pH)_{B=c}$, Figure 16, along pH axis was found graphically, and curves were made to coincide by replotting the data as a function of

$$x = \log B + t \cdot \log h^{-1} \qquad (42)$$

In order to find out precise value of 't', various values of 't' were chosen (integral as well as nonintegral), such that the values of 'x' calculated for a series of B (aluminium ion concentrations used) values showed minimum difference. The value of 't' thus obtained was '2.611', and corresponding values of x are given in Table 7 in Appendix I. The complex may then be represented as a compounds $B\left[(H)_{-st}\ Bs\right]_l$ of core B with l links $\left[(H)_{-st}\ Bs\right]$, where 's' is smallest integer (Rossotti, and Rossotti (1956)) such that the product st is also integral, and l may have a series of integral values upto a maximum of L, which is six in case of aluminium ion. But as present system indicates presence of unique complex $B_Q(H)_{-P}$ which can be represented in terms of core-complex as $B\left[Bs(H)_{-st}\right]_L$.

With the assumption that activity coefficients are effectively constant, the concentration of core complex formed can be represented by

$$(1 + sL).B \rightleftharpoons B\left[Bs(OH)_{st}\right]L + L.st.H^{+} \qquad (43)$$

The concentration of $B\left[Bs(H)_{-st}\right]L$ will be given by

$$B\left[Bs(H)_{-st}\right] = K_L \cdot b \cdot u^{Ls} \qquad (44)$$

where K_L is equilibrium constant for reaction (43), and u is convenient auxiliary variable defined by

$$u = b(h)^{-t} \qquad (45)$$

The concentration of bound (H) and of total B are given respectively by

$$B = b + (1 + sL)\ B(Bs(H)_{-st})_L \qquad (46)$$

$$= b(1 + ug' + g)$$

where $g = K_L \cdot u^{Ls}$

$$g' = K_L \cdot u^{Ls-1}.Ls.$$

$$h - H - Kw.h^{-1} = b.t\ ug' \qquad (47)$$

Identification of unique species and determination of stability constants was done by direct comparison of the

experimental data $\bar{n}(pH)_{B=c}$ with appropriate normalised curves. Normalised curves were obtained as follows.

Combination of equations (46), and (47)

$$y = \frac{\bar{n}}{t} = \frac{h - H - Kw.h^{-1}}{B.t} = \frac{b.t.ug'}{b(1 + ug' + g)t}$$

$$= \frac{sLg}{1 + (1 + sL)g} \qquad (48)$$

and $$x = \log B + t \log h^{-1} \qquad (49)$$

$$= \frac{1}{sL} \log g - \frac{1}{s} \log R + \log\left[1 + (1 + sL)g\right] \qquad (50)$$

where $$L \log R = \log K_L = \log x_{qp} \qquad (51)$$

The normalised abscissa is chosen, therefore, as

$$X = x + \log 2 - x_{\frac{1}{2}} \qquad (52)$$

where $x_{\frac{1}{2}}$ is the value of x, for $y = \frac{1}{2}$

when $y = \frac{1}{2}$, whence by substitution in equation (50)

$$x_{\frac{1}{2}} = \log 2sL - \frac{1}{s} \log R - \frac{1 + sL}{sL} . \log(sL - 1) \qquad (53)$$

Combining equations (50), (52), and (53)

$$X = \frac{1}{sL} \log g + \log\left[1 + (1 + sL)g\right] - \log sL + \frac{1 + sL}{sL} \log(sL - 1) \qquad (54)$$

using equations (48), and (54) normalised curves were plot for different values of 'sL' and compared with plot obtained from experimental results. Normalised curve for $sL = \infty$ seems to coincide with y(X) curve obtained from the results, Figure 18, thus indicating to the precipitation of $Bs(H)_{-st}$ in the absence of soluble complexes. In the present case s = 13, and st = 13x2.611 = 33.943, i.e. approximately 34. Therefore predominant species formed are $Al_{13}(OH)_{34}$, and these precipitate in the presence of SO_4^{--}. Sillen (1959) has mentioned the presence of $Al_{13}(OH)_{32}$ species in some basic aluminium salt crystal (Johanson (1966)), as a possibility and still more recently (Sillen (1962)), a mixture of $Al_{13}(OH)_{34}$, and $Al_7(OH)_{17}$. Present investigation shows that predominant species in presence of SO_4^{--} ions is $Al_{13}(OH)_{34}$, which further precipitates, and therefore theoretically speaking composition of precipitate should be $Al_{13}(OH)_{34}(SO_4)_{2.5}$. The composition of precipitate found from chemical analysis was $Al(OH)_{2.4}\cdot(SO_4)_{0.3}$. The difference may be due to limiting accuracy in estimation of Al(III) in solution.

The stability constants K obtained from the Figure 19 is $10^{-7.17}$. Therefore $K_{13,34} = \left[10^{-7.17}\right]^{13} = 10^{-93.21}$. Taking into consideration the activity coefficient, $K_{13,34} = 10^{-96.71}$, whereas the value of $K_{13,34}$ available in literature is $10^{-97.22}$.

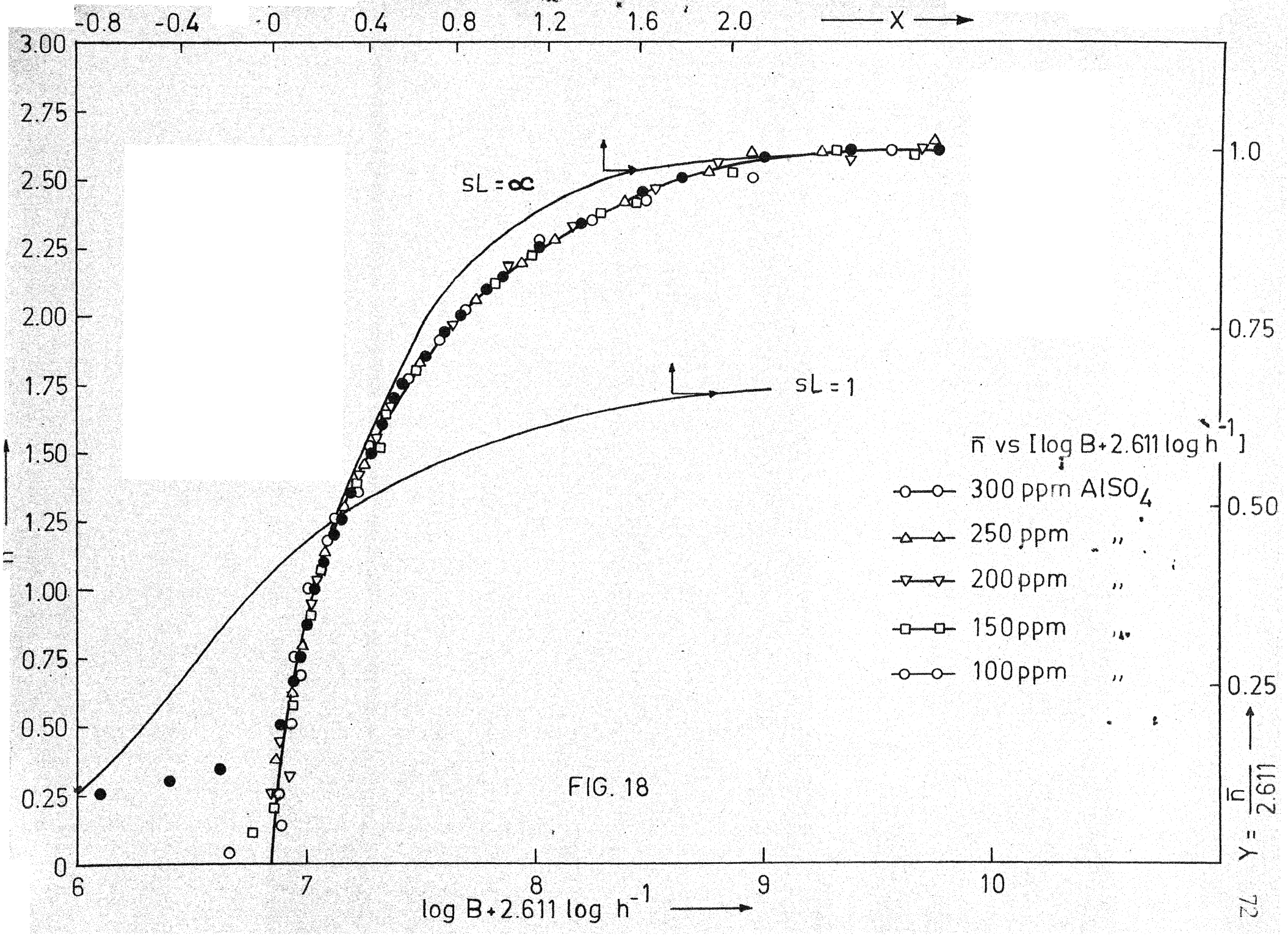

-0.8
-0.4
0
0.4
0.8
1.2
1.6
2.0
X
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0
sL = ∞
sL = 1
1.0
0.75
0.50
0.25
n̄ vs [log B+2.611 log h⁻¹]
300 ppm AlSO4
250 ppm ,,
200 ppm ,,
150 ppm ,,
100 ppm ,,
6
7
8
9
10
log B+2.611 log h⁻¹
Y = n̄/2.611

FIG. 18

The region (ii), $2.6 \leqslant \bar{n} \leqslant 3$, and $5.1 \leqslant pH \leqslant 7.0$, curves are coincident (Figure 16), thus indicating the presence of homonuclear species. The $\bar{n}$ values almost linearly vary with pH, and are independent of aluminium ion concentration. The log B(pH)$_{\bar{n}=c}$ curves, Figure 17, show set of vertical lines beyond $\bar{n} = 2.61$, therefore

$$\left(\frac{\log h^{-1}}{\log B}\right)_{\bar{n} \gg 2.6} = 0 \qquad (55)$$

when $5.1 \leqslant pH \leqslant 7.0$

since

$$\bar{r} = \frac{\sum_{1}^{Q}\sum_{0}^{P} B_q (H)_{-p}}{B} = r_i + \int_{o}^{a}\left[\left(\frac{\log h^{-1}}{\log B}\right) - \bar{n}=c\right] d\bar{n}_{B=c} \qquad (56)$$

$$= \text{Constant.} \qquad \text{when} \quad 5.1 \leqslant pH \leqslant 7.0.$$

Most of aluminium ions precipitate near pH = 7.0, and therefore

$$\bar{r} = 0 \qquad pH = 7$$

Hence

$$\therefore \log \frac{B}{b} = \int_{B=c} \bar{n}\, d \log h^{-1} + 1 - \bar{r} \qquad (57)$$

reduces to

$$\log \frac{B}{b} = \int_{B=c} \bar{n} \cdot d \log h^{-1} + 1 \qquad (58)$$

$\int_{B=c} \bar{n}.d \log h^{-1}$ = Area under the curve of $\bar{n}(pH)_{B=c}$ curves.

The area was calculated using Simpson's rule. The calculated areas at each pH value are given in Tables 1 to 6 in Appendix I. The solubility product (K_{so}), calculated from the experimental data is $10^{-32.28 \pm 0.02}$, taking into consideration activity coefficients, the above value reduces to $K_{so} = 10^{-32.8 \pm 0.02}$, whereas value of solubility product available from literature (Sillen, and Martell (1971)) is 10^{-33}. The value of K_{so} calculated from the data is in close agreement with the standard available value.

Since $\bar{n}$ values linearly vary with pH, and $\bar{r} = 0$ in the range $5.1 \leqslant pH \leqslant 7.0$, therefore soluble Al^{3+} concentration can be found out with the help of K_{so} calculated. In the range $5.1 < pH < 7.0$, the composition of precipitate varies linearly from $Al_{13}(OH)_{34}(SO_4)_{2.5}$ to $Al(OH)_3$. This shows that beyond pH 5.1, SO_4^{--} are continually replaced by OH^- ions and this replacement completes in the vicinity of pH 7.

The $\bar{n}(pH)_{B=c}$ curve, Figure 16, for $Al^{3+} = 1.57 \times 10^{-4}$ (50 ppm $Al:SO_4$) is entirely different from those for higher concentrations, but is parallel to them till $\bar{n} = 1.25$. The same solution showed late precipitation when compared to

solutions with higher concentration of aluminium. This may be due to following reasons:

1. The rate of precipitation depends on concentration of polynuclear species and rate of interaction between polynuclear species and SO_4^{--}. Relative concentration of polynuclear species to SO_4^{--} if ever remains same, in case of all solution, but concentration of polynuclear species and SO_4^{--} ions is different and lowest in case of solution with Al(III) = 1.57×10^{-4}. This will result into lower rate of precipitation.
2. The predominant species present at higher concentration of aluminium is $Al_{13}(OH)_{34}$. Polynuclear complex formation is triggered by the concentration of monomers, giving rise to primary hexagonal units. Further condensation of hexagonal units is controlled by charge on the primary unit formed. This condensation will be accelerated in the presence of SO_4^{--} attached to the primary hexagonal unit, and also concentration of primary hexagonal units. Therefore at low concentration of Al - 1.57×10^{-4} M, due to lower concentration of SO_4^{--} and primary hexagonal units, most of them must be staying in solution phase in the form of $Al_7(OH)_{17}$ and part of it must be precipitating.
3. The pH range in which $Al_{13}(OH)_{34}$ species is predominant in case of higher concentrations, whereas in case of Al(III) concentration 1.57×10^{-4}, $Al_7(OH)_{17}$ might be a predominant species.

6.2 Study of aluminium protein interaction

6.2.1 Potentiometric titration of protein solution

Potentiometric titrations were performed with bovine serum albumin solution against NaOH and HCl so as to determine quantitatively the number of different ionogenic groups present as side chains on the protein molecules. The results of protein titration are shown in Fig. 19, and are presented in a tabular form in Appendix II, Table 1A. The groups present on protein molecules dissociate in different pH ranges. Cannan (1942) proposed following classification for different titration ranges.

1. The titration from maximum bound ion value, in the region of pH 1.5, to that at pH 6 is attributed to the ionization of side chain carboxyl groups.

2. The titration between pH 6 and 3.5 is attributed to histidine and terminate α-amino groups.

3. The titration from pH 8.5 to the maximum at pH 11-12 is attributed to ε-amino groups of lysine, phenolic hydroxyl groups of tyrosine, and sulphydryl groups of cysteine.

Aluminium ions are classified as hard Lewis Acids. In general hard acids prefer to coordinate with hard bases to which aluminium ions are no exceptions. Aluminium ions show affinity towards hard bases like F^-, OH^-, RCOOH, ROH incomparison to soft bases like RNH_2, RHS, and RS.

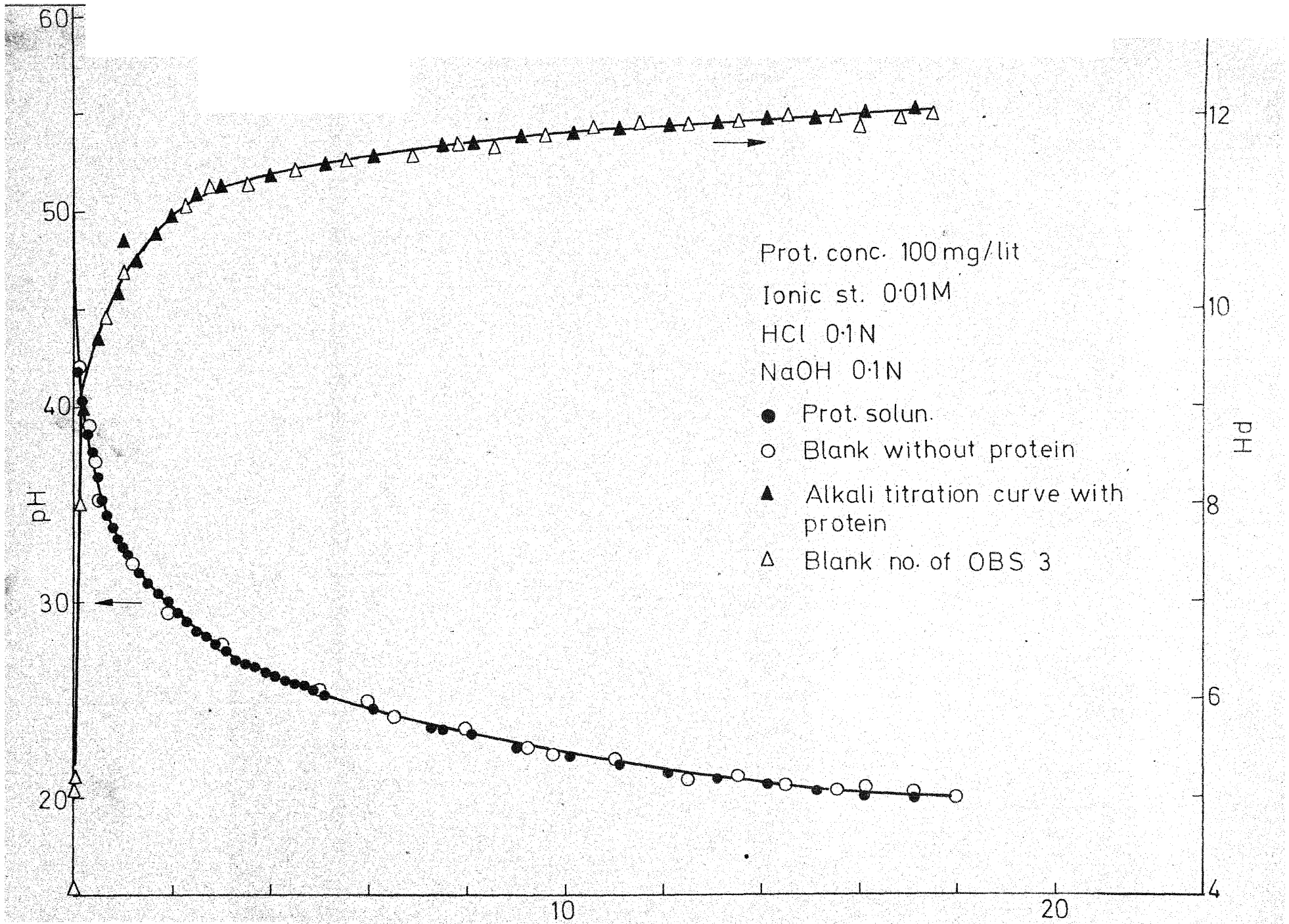


FIG.19 PROTEIN TITRATION

Therefore in present discussion more emphasis is laid on carboxyl groups. It is evident from Table 1A that the difference in corresponding pH values for blank and protein solution is almost negligible beyond pH 2.790. In order to obtain hydrogen ion uptake by carboxyl groups, corresponding hydrogen ion concentration of blank was subtracted from that of protein solution. The calculated values are presented in Table 1B [Appendix II]. The hydrogen ion uptake should increase with reduction in pH values and attain a maxima in the vicinity of pH 1.5 to 2. The calculated values do not exhibit any specific trend in order to calculate dissociation constant for carboxyl groups. This may be because of errors involved in pH measurements. The errors may be due to (i) limited accuracy of instrument, (ii) old glass electrode, and (iii) interference due to adsorption of proteins on glass membrane.

Maximum hydrogen ion uptake by carboxyl groups is found to be $10^{-3.6372}$M per 100 mg of bovine serum albumin. Kirschenbaum (1971) has analytically obtained the amino acid composition of bovine serum albumin. Using Kirschenbaum's data the concentration of carboxyl groups was calculated and is equal to $10^{-3.70}$M per 100 mg of bovine serum albumin. The above value is quite in close agreement with the value obtained from experimental results. But even after several repetitions of protein titration no specific trend in hydrogen ion uptake with pH could be obt ined for bovine serum albumin.

6.2.2 Kinetics of aluminium protein interaction

The results are given in Table 2 [Appendix II]. It is evident from the results that the amount of protein (Bovine serum albumin) in the supernatant remains almost constant after 15 minutes. The minimum time required for centrifuging the drawn sample being 15 minutes, no intermediate sample could be analysed. The aggregation of bovine serum albumin molecules can be assumed to be a two step reaction..

i) Reaction between aluminium ions and individual protein molecules.

ii) Interaction between reacted protein molecules resulting in their aggregation.

The results obtained clearly show that aggregation of protein (BSA) is almost complete within initial 15 minutes. This clearly indicates that binding of aluminium ions to protein molecules is a fast process. In that case the amount of protein aggregated will not be a function of engineering parameters like intensity of mixing. As no information could be obtained at lesser time interval about protein concentration in the supernatant, nothing can be said about aggregation kinetics.

Protein solutions exhibit a distinct absorption band at 280 mμ. The aggregation kinetics was studied with the help of spectrophotometer and results obtained are given

in Table II [Appendix II]. The absorbance show a constant value after about 4 to 6 minutes. Therefore it can be positively said that the aggregation of protein molecules is completed within initial 6 minutes. The absorbance values being low of the order of 0.08 at protein (BSA) concentration 100 mg per litre, repetability in measurement of absorbance with time was poor.in successive trials. From the above results obtained it can be concluded that binding of aluminium ions to protein molecules resulting into aggregation of protein molecules is a fast process.

6.2.3 Reaction equilibrium

In order to establish the type of interaction existing between proteins and aluminium ions the equilibrium pH of aluminium-protein solution, and only aluminium solution was observed at very close range of $[\frac{NaOH}{Al}]$ molar ratios. The results for bovine serum albumin, and casein are shown in Figs. 20, 21 respectively. The same results are presented in a tabular form in Table 3 and 4 [Appendix II]. It is clear enough from Figs. 20, 21 that after about pH 5 curves are converging in case of bovine serum albumin, and almost parallel in case of casein. For the same $[\frac{NaOH}{Al}]$ molar ratio the equilibrium pH attained by aluminium-protein solution is hi_her when compared to aluminium solution. This indicates that aluminium ions are reacting with carboxyl and other groups present on protein molecules. The change in

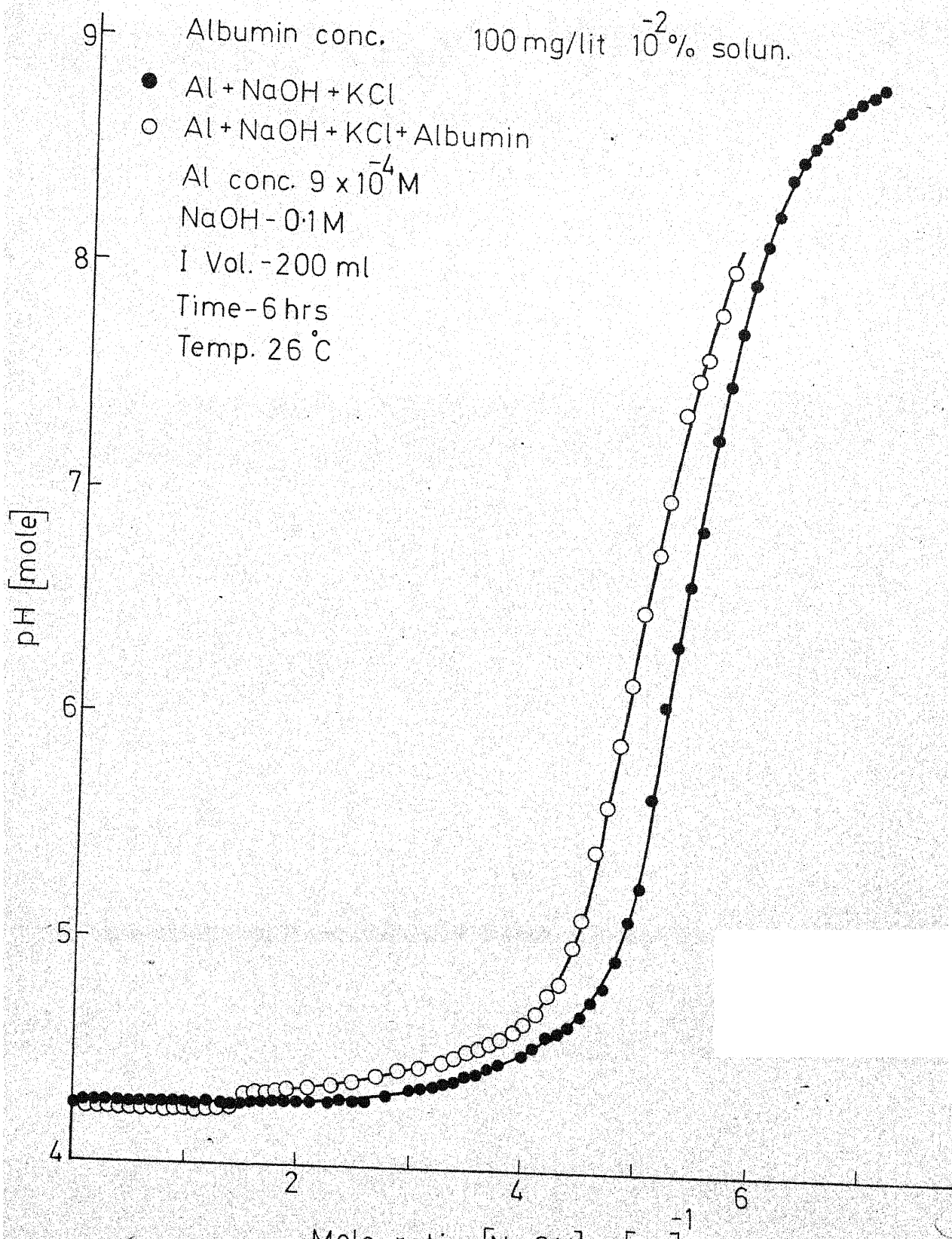


FIG. 20 Al-ALBUMIN INTERACTION

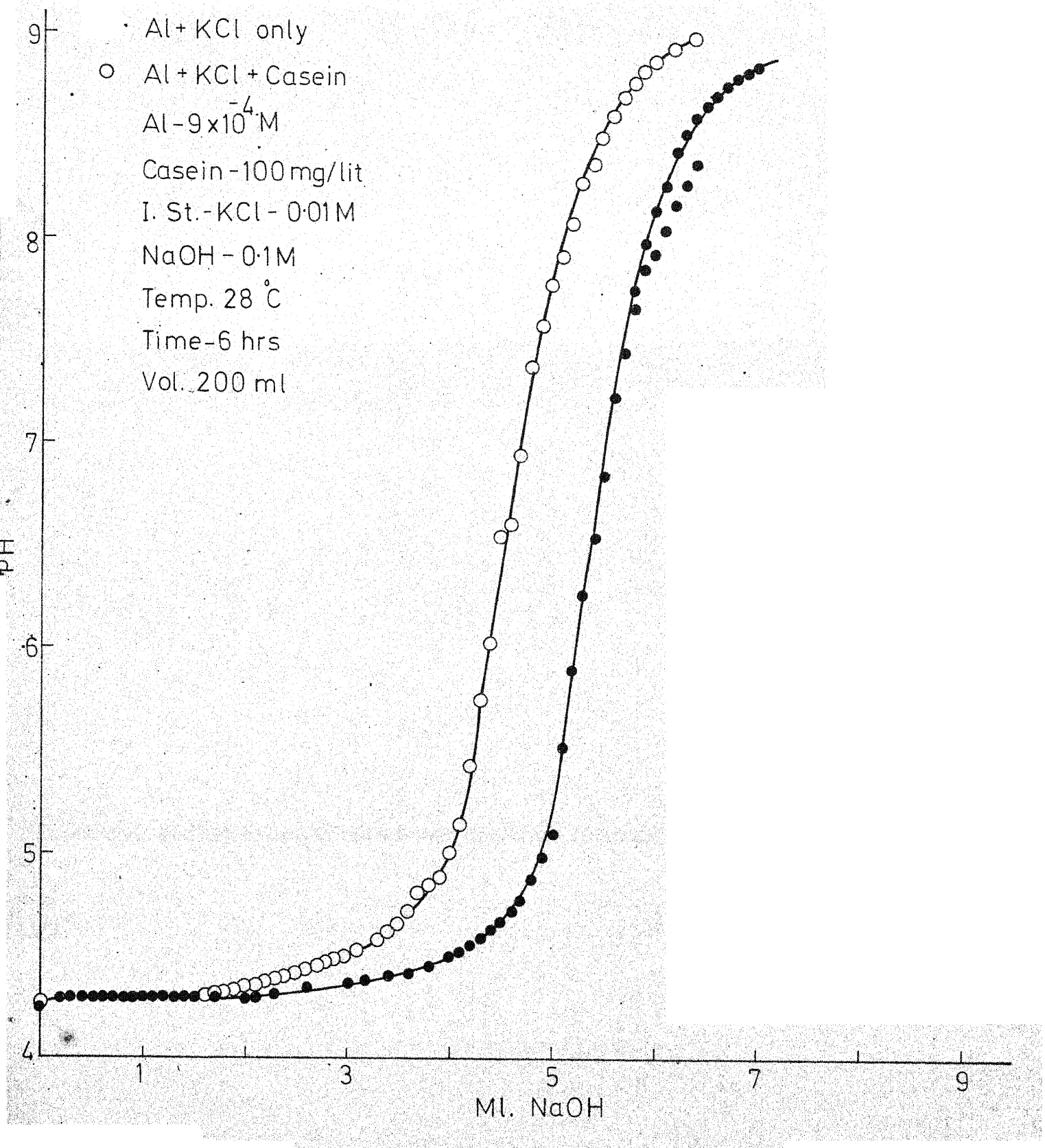


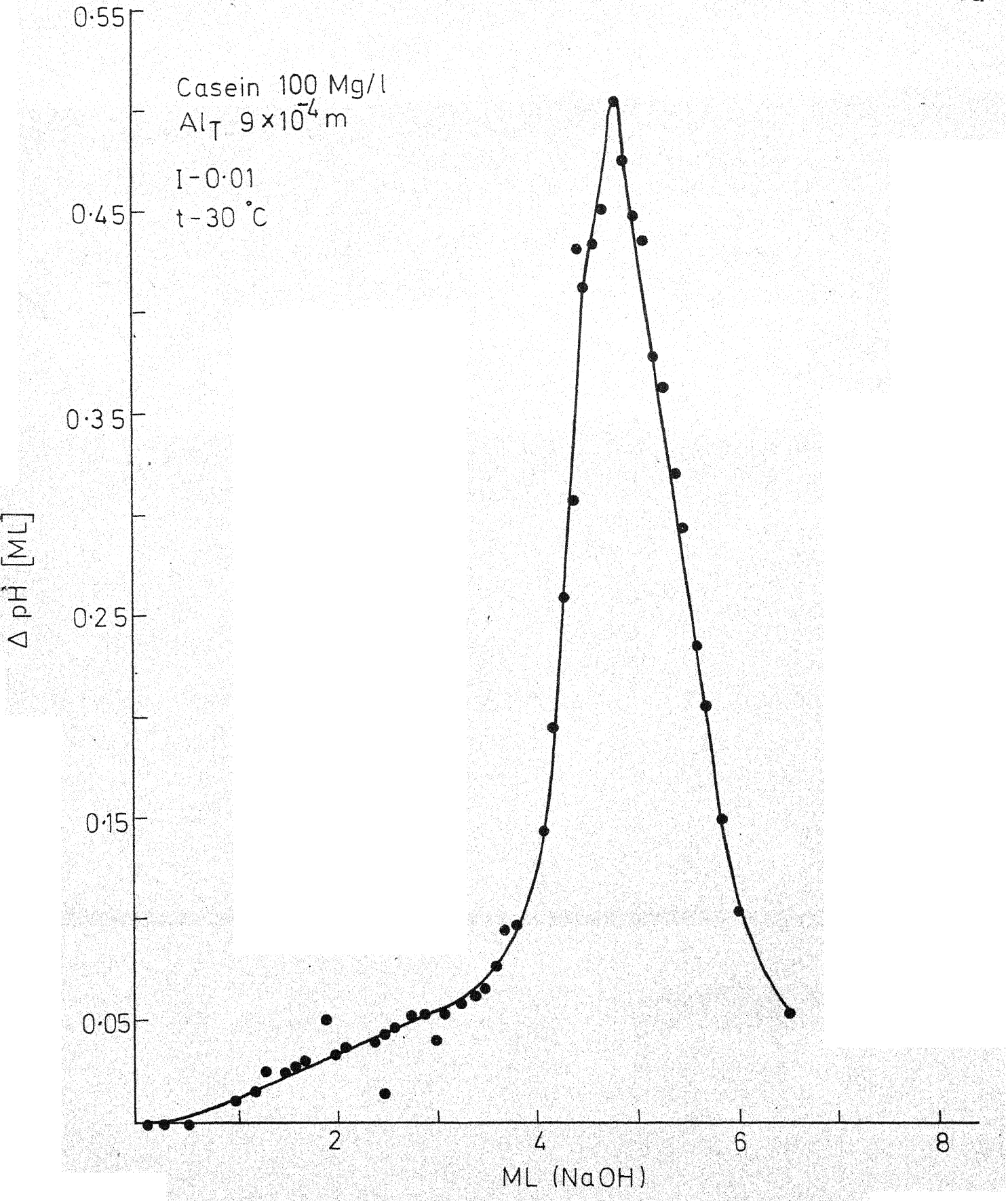
FIG. 21 Al CASEIN INTERACTION

pH per ml NaOH added against [$\frac{NaOH}{Al}$] molar ratio is shown in Fig. 22 for casein in order to bring out the magnitude of change in pH due to reaction between aluminium ions and casein The isoelectric point for bovine serum albumin is around pH 4.9. The aggregation of proteins below pH 4.9 should be because of reaction between aluminium ions and protein molecule the reacted aluminium ions must be serving as a point of contact with other protein molecules leading to formation of clasters of protein molecules. From the data obtained it would have been possible to calculate amount of aluminium ions reacted if proper information about number of carboxyl groups and their dissociation constant would have been available from protein titrations carried out.

Qualitatively the amount of aluminium ions reacted with protein molecules can be found out as follows. At pH 7 aluminium ions are predominantly present as $Al(OH)_3$ species. In order to reach pH 7 the amount alkali required is different for aluminium-BSA, and aluminium-casein solutions when compared to aluminium solution only. The difference is 0.3 ml NaOH for former and 0.85 ml NaOH for later one. This amounts to 1.5×10^{-4}M and 4.25×10^{-4}M of OH. As predominant species are $Al(OH)_3$, and Al^{3+},

$$\text{The amount of } Al^{3+} \text{ reacted} = \frac{\text{excess conc. of OH}}{3}$$

$$\text{The amount of } Al^{3+} \text{ reacted with albumin} = 0.5 \times 10^{-4}\text{M.}$$



FIG·22 CHANGE IN pH PER ML. NaOH ADDED

as coordination No. for aluminium is 6.

It can be assumed that all possible sites can never be occupied due to their positions on protein molecules in that case at pH 7, the amount of aluminium ions reacted should the be less than the above two limits. The amount of aluminium ions reacted is 0.5×10^{-4} M in case of bovine serum albumin. If all sites on protein (BSA) are occupied and aluminium is bound to 4 carboxyl groups each then

$$\text{the amount aluminium ions reacting with BSA} = \frac{\text{Total No. of carboxyl groups}}{4}$$

$$= \frac{2.0614 \times 10^{-4}}{4}$$

$$= 0.5 \times 10^{-4} \text{ M approximately.}$$

But all sides can never be bound by same coordinations number. Therfore the caluclated value from the results should be smaller than 0.5×10^{-4} M, but it is not so. Therefore the mechanism of protein aggregation must be different at pH > isoelectric point. In this range proteins exhibit negative charge, and the interaction with aluminium ions may be of similar nature when compared to hydrophobic colloids. But chemical affinity towards carboxyl groups cannot be discarded. Hence both chemical, and electrostatic binding may be the reason for protein aggregation.

The curves in Fig. 21 are almost parallel to each other beyond pH 5, and thus indicate that constant amount of aluminium ions remain bound to casein molecules, whereas the curves in Fig. 20 are converging and hence the amount of aluminium ions bound to bovine serum albumin molecules must be decreasing with pH.

CHAPTER VII

CONCLUSIONS AND SUGGESTIONS

7.1 Conclusions

The results obtained in the present investigation leads to the following conclusions:

(1) Precipitation of aluminium sulphate was found to be complete at pH 4.5. The precipitate composition was $Al(OH)_{2.4}(SO_4)_{0.3}$. Concentration of polymeric species was found to be low, as $(\frac{OH}{Al})$ molar ratio in the supernatant was extremely less.

(2) Increase in sulphate ion concentration did not affect aluminium hydrolysis equilibrium. Similarly, different ionic medium such as potassium chloride did not affect aluminium hydrolysis equilibrium and hence equilibrium products.

(3) Casein showed greater binding capacity for aluminium, in comparison to bovine serum albumin. The amount of aluminium bound to casein remained constant upto pH 8.0, whereas, in case of bovine serum albumin, it reduced with pH of the solution. Aluminium ions seems to form co-ordination complex with number of carboxyl groups simultaneously.

(4) Calcium and magnesium increased the amount of proteins precipitating from the solution. This was true in the case of casein, but not in the case of borine serum albumin.

(5) In the presence of diprotic acids like oxalic and malic acid, borine serum albumin showed a reduction in the amount of bound aluminium ions whereas casein showed a marginal change.

7.2 Suggestions

(1) In order to find out the composition of precipitate it was assumed that the precipitate is electroneutral. The early precipitate formation in the presence of SO_4^{--} has been observed by several research workers. This precipitate, even after aging for several days, has shown amorphus nature. This indicates that SO_4^{--} ions are forming outer sphere complexes. In that case the validity of the assumption made demands substantial proof. This can be achieved by estimating SO_4^{--} concentration in the supernatant.

(2) In the present investigation it has been found out that the hydrolysis of aluminium ions in the presence of anions like SO_4^{--}, leads to the formation of only one predominant species $Al_{13}(OH)_{34}$ and not a series of core

complexes. In order to generalize the present finding it is necessary to carry out aluminium hydrolysis experiments with aluminium concentration as high as 10^{-1} M and as low as 10^{-6} M.

(3) It was not possible to correlate change in pH with the amount of aluminium ions reacted with protein due to considerable variation in the data obtained. It seems to be possible with the use of higher concentration of proteins in the experiment.

(4) Correlation should be established between the amount of aluminium ions reacted with protein and protein precipitated.

(5) I.R. spectral studies of the aggregated proteins should be conducted in order to confirm binding sites and coordination.

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Appendix I

$Al\text{-}SO_4$ Hydrolysis Equilibrium Data

TABLE - 1.

Total Al conc. - 1.57×10^{-4} M. (50 ppm $Al_2(SO_4)_3$)

Ionic st. - 0.01 (KNO_3)

Alkali used - KOH (0.02 M)

Temp. - 24°C to 28°C

Total vol. - 200 ml.

ml. KOH added	pH of the supernatant after				$\bar{n}$	Conductivity m mho.	Turbidity (FTU) after 2 Hrs.
	2 Hrs.	12 Hrs.	24 Hrs.	72 Hrs.			
B	4.20	4.08	4.05	4.05	-	1.70	0.60
0.2	4.21	4.14	4.11	4.11	0.065	1.70	0.60
0.5	4.22	4.15	4.12	4.11	0.234	1.65	0.60
1.0	4.24	4.20	4.18	4.15	0.541	1.65	0.60
1.5	4.28	4.20	4.20	4.18	0.883	1.65	0.65
1.8	4.28	4.25	4.20	4.20	0.989	1.65	0.70
2.0	4.30	4.28	4.25	4.25	1.089	1.60	0.80
2.2	4.35	4.32	4.30	4.30	1.213	1.60	0.80
2.5	4.40	4.35	4.31	4.32	1.374	1.60	0.90
2.8	4.40	4.35	4.35	4.33	1.534	1.60	0.90
3.0	4.42	4.40	4.39	4.40	1.627	1.60	1.00
3.5	4.50	4.48	4.46	4.45	1.897	1.60	1.00
3.8	4.60	4.58	4.58	4.54	2.035	1.65	1.20
4.0	4.70	4.65	4.65	4.65	2.132	1.65	1.30
4.5	5.10	5.28	5.40	5.40	2.333	1.70	1.50
5.0	6.20	5.10	6.09	6.10	2.623	1.70	1.50
5.5	7.13	6.85	6.70	6.70	2.930	1.75	1.50

TABLE - 2.

Total Al conc. - 4.58×10^{-4} M (100 ppm. $Al_2(SO_4)_3$)

Ionic st. - 0.01 (KNO_3)

Alkali used - KOH (0.04 M)

Temp. - 24°C to 28°C

Total vol. - 200 ml.

ml. KOH	pH of supernatant after				$\bar{n}$	Conductivity after 24 Hrs. m mho.	Turbidity after 3 Hrs. (FTU)	$\bar{n}$.dpH
	2 Hrs	12 Hrs	24 Hrs	72 Hrs				
B	4.05	4.00	3.95	3.95	-	1.70	0.2	-
0.2	4.08	4.00	3.98	3.98	0.103	1.65	0.9	0.001552
0.5	4.10	4.00	3.99	3.98	0.287	1.60	1.5	0.001552
0.8	4.10	4.00	3.99	3.99	0.478	1.55	2.00	0.0035
1.0	4.11	4.01	4.00	4.00	0.598	1.55	2.50	0.0089
1.2	4.11	4.01	4.01	4.01	0.718	1.55	3.00	0.01546
1.5	4.12	4.02	4.02	4.01	0.902	1.60	3.00	0.02356
1.8	4.16	4.06	4.05	4.05	1.072	1.60	3.50	0.05319
2.0	4.18	4.08	4.08	4.07	1.181	1.60	4.00	0.08699
2.2	4.19	4.10	4.09	4.08	1.302	1.60	4.50	0.0994
2.5	4.21	4.12	4.11	4.12	1.482	1.60	4.50	0.1273
3.8	4.27	4.20	4.18	4.16	1.636	1.60	5.00	0.2364
3.0	4.30	4.16	4.20	4.19	1.753	1.60	5.50	0.2702
3.2	4.35	4.30	4.24	4.24	1.864	1.60	6.00	0.3426
3.5	4.39	4.28	4.30	4.29	2.031	1.60	6.75	0.4594
3.8	4.50	4.40	4.40	4.40	2.189	1.60	7.50	0.6705
4.0	4.58	4.50	4.42	4.43	2.310	1.60	7.50	0.7155
4.2	4.78	4.70	4.62	4.60	2.394	1.65	7.40	1.1859
4.5	5.19	5.22	4.910	4.92	2.547	1.65	7.40	1.9023
4.8	5.91	5.80	5.710	5.70	2.705	1.70	6.20	4.0035
5.0	6.39	6.38	6.190	6.20	2.829	1.70	6.60	5.3593
5.2	6.83	6.30	6.25	6.22	2.955	1.70	5.60	5.5328
5.5	7.49	7.35	7.05	7.05	3.145	1.70	4.60	7.9737

TABLE - 3.

Total Al(III) conc. - 4.711×10^{-4} M (150 ppm. $Al_2(SO_4)_3$)

Ionic st. - 0.01 (KNO_3)

Alkali used - 0.06 M (KOH)

Temperature - 24°C to 28°C

Total vol. - 200 ml.

KOH added ml.	pH of supernatant after				$\bar{n}$	Conductivity after 24 Hrs. m mho.	Turbidity after 2 Hrs (FTU)	$\bar{n}$.dpH
	2 Hrs	12 Hrs	24 Hrs	72 Hrs				
B	3.95	3.90	3.85	3.85	-	1.70	0.00	-
0.20	3.95	3.92	3.88	3.88	0.107	1.65	0.80	0.0016
0.50	3.98	3.90	3.89	3.89	0.292	1.70	1.50	0.0036
0.80	4.00	3.90	3.89	3.89	0.483	1.70	2.20	0.0046
1.00	4.00	3.92	3.90	3.90	0.604	1.65	2.50	0.0099
1.20	4.01	3.95	3.91	3.91	0.725	1.65	3.00	0.01663
1.50	4.02	3.95	3.92	3.92	0.910	1.60	3.00	0.02481
1.80	4.02	3.95	3.95	3.94	1.080	1.65	3.50	0.05466
2.00	4.00	4.00	3.99	3.99	1.190	1.60	4.00	0.1000
2.20	4.00	4.00	4.00	4.00	1.310	1.60	4.00	0.1126
2.50	4.10	4.05	4.02	4.01	1.490	1.60	5.00	0.1406
2.80	4.10	4.10	4.10	4.08	1.652	1.60	5.00	0.2662
3.00	4.11	4.11	4.12	4.11	1.771	1.60	6.0	0.3004
3.20	4.15	4.16	4.16	4.14	1.948	1.70	6.50	0.3747
3.50	4.20	4.21	4.23	4.22	2.053	1.65	6.50	0.5149
3.80	4.30	4.30	4.31	4.30	2.224	1.60	7.00	0.6860
4.00	4.32	4.33	4.33	4.34	2.347	1.65	7.00	0.7317
4.20	4.52	4.54	4.54	4.52	2.435	1.70	8.00	1.2338
4.50	4.81	4.82	4.82	4.81	2.598	1.75	8.50	1.9382
4.80	5.80	5.81	5.83	5.82	2.761	1.80	7.80	4.6446
5.00	6.50	6.45	6.30	6.28	2.885	1.85	6.00	5.9715
5.20	7.15	7.10	7.00	6.97	3.011	1.80	5.80	8.03519

TABLE - 4.

Total Al(III) conc. - 6.281×10^{-4} M (200 ppm. $Al_2(SO_4)_3$)

Ionic st. - 0.01 (KNO_3)

Alkali used - KOH (0.08 M)

Temperature - 24 to 28°C

Total vol. - 200 ml.

ml. KOH added	pH of supernatant after				$\bar{n}$	Conductivity after 24 Hrs. m mho.	Turbidity after 2 Hrs. (FTU)	$\bar{n}$.dpH
	2 Hrs	12 Hrs	24 Hrs	72 Hrs				
B	3.95	3.90	3.80	3.79	-	1.80	0.30	-
0.25	3.95	3.90	3.80	3.80	0.1591	1.80	1.00	-
0.50	3.98	3.90	3.85	3.80	0.2909	1.75	1.80	0.0192
0.75	4.00	3.95	3.85	3.82	0.4501	1.75	2.00	0.0192
1.00	4.01	3.90	3.85	3.82	0.6093	1.70	2.00	0.0192
1.25	4.01	3.90	3.875	3.85	0.7560	1.70	2.80	0.0363
1.50	4.02	4.00	3.90	3.90	0.9032	1.70	3.50	0.0363
1.75	4.01	4.00	3.90	3.91	1.0624	1.70	4.00	0.0363
2.00	4.01	4.00	3.900	3.920	1.2216	1.70	4.30	0.0610
2.25	4.00	4.00	3.95	3.95	1.3590	1.70	4.80	0.1255
2.50	4.00	4.00	4.00	4.00	1.4987	1.70	5.00	0.1970
2.75	4.02	4.02	4.025	4.025	1.6490	1.70	5.60	0.2363
3.00	4.05	4.05	4.05	4.05	1.7998	1.70	5.60	0.2794
3.25	4.10	4.12	4.11	4.11	1.9510	1.70	6.00	0.3263
3.50	4.12	4.12	4.13	4.16	2.0956	1.70	7.60	0.4274
3.75	4.25	4.25	4.24	4.24	2.2359	1.70	8.50	0.5899
4.00	4.31	4.32	4.320	4.32	2.3701	1.75	10.00	0.8855
4.25	4.63	4.63	4.650	4.650	2.4939	1.75	12.20	1.5543
4.50	5.25	5.27	5.270	5.28	2.6231	1.80	11.80	3.0894
4.75	5.80	5.80	5.80	5.80	2.7748	1.85	10.70	4.7088
5.00	6.75	6.50	6.40	6.41	2.9321	1.90	9.00	6.4208
5.25	7.10	7.00	7.00	7.01	3.02714	1.90	8.50	8.2086

TABLE - 5.

Total Al(III) conc. - 7.852×10^{-4} M (200 ppm. $Al_2(SO_4)_3$)

Ionic st. - 0.01 (KNO_3)

Alkali used - KOH (0.1 M)

Temperature - 24 to 28°C

Total vol. - 200 ml.

ml. KOH added	pH of supernatant after				$\bar{n}$	Conductivity after 24 Hrs. m mho.	Turbidity after 2 Hrs. (FTU)	$\bar{n}$.dpH
	2 Hrs	12 Hrs	24 Hrs	72 Hrs				
B	3.90	3.82	3.75	3.75	-	1.85	0.20	-
0.25	3.90	3.83	3.78	3.78	0.1440	1.80	1.80	0.00216
0.50	3.90	3.85	3.80	3.80	0.2940	1.80	2.50	0.0065
0.75	3.90	3.85	3.82	3.82	0.444	1.75	3.50	0.01391
1.00	3.90	3.88	3.83	3.83	0.599	1.75	4.00	0.01912
1.25	3.90	3.88	3.84	3.83	0.753	1.75	4.80	0.02589
1.50	3.91	3.91	3.855	3.85	0.906	1.70	5.00	0.03834
1.75	3.92	3.90	3.870	3.87	1.060	1.70	5.00	0.05308
2.00	3.95	3.90	3.880	3.88	1.215	1.65	6.00	0.06464
2.25	4.00	3.98	3.950	3.95	1.349	1.65	6.80	0.1544
2.50	4.00	3.98	3.970	3.97	1.502	1.65	7.00	0.1829
2.75	4.00	4.00	3.990	3.99	1.655	1.65	7.20	0.2145
3.00	4.02	4.02	4.030	4.03	1.803	1.65	8.00	0.2836
3.25	4.08	4.08	4.09	4.08	1.947	1.65	9.00	0.3961
3.50	4.16	4.15	4.16	4.15	2.090	1.65	11.20	0.5374
3.75	4.24	4.25	4.25	4.23	2.233	1.65	12.40	0.7319
4.00	4.33	4.32	4.33	4.32	2.380	1.65	13.50	0.9164
4.25	4.55	4.56	4.55	4.56	2.516	1.65	13.00	1.4550
4.75	5.46	5.45	5.45	5.47	2.643	1.80	11.80	3.7766
5.00	6.30	6.20	6.20	6.18	2.799	1.90	9.30	5.8175
5.25	7.10	7.05	6.90	6.82	2.957	1.95	8.00	7.8323

TABLE - 6.

Total Al(III) conc. - 9.422×10^{-4} M (300 ppm. $Al_2(SO_4)_3$)

Ionic st. - 0.01 (KNO_3)

Alkali used - KOH (0.1 M)

Temperature - 24 to 28°C

Total vol. - 200 ml.

ml. KOH added	pH of supernatant after				$\bar{n}$	Conductivity after 24 Hrs. m mho.	Turbidity after 2 Hrs. (FTU)	$\bar{n}$.dpH
	2 Hrs	12 Hrs	24 Hrs	72 Hrs				
B	3.70	3.91	3.70	3.710	-	1.90	0.0	-
0.25	3.73	3.95	3.75	3.750	0.1096	1.90	2.0	-
0.50	3.75	4.00	3.75	3.760	0.2420	1.85	3.0	0.0060
0.75	3.76	4.02	3.77	3.776	0.3644	1.80	3.0	0.0136
1.00	3.78	4.05	3.80	3.800	0.4870	1.80	3.5	0.0243
1.25	3.79	4.04	3.810	3.800	0.6152	1.80	3.5	0.0309
1.50	3.80	4.05	3.825	3.800	0.7429	1.75	3.0	0.0397
1.75	3.82	4.07	3.825	3.815	0.8760	1.75	3.5	0.0397
2.00	3.85	4.08	3.850	3.850	0.999	1.70	4.0	0.0632
2.25	3.87	4.09	3.875	3.870	1.123	1.70	4.0	0.0897
2.50	3.90	4.10	3.900	3.900	1.248	1.70	4.5	0.1194
2.75	3.90	4.15	3.925	3.925	1.374	1.70	5.0	0.1521
3.00	3.95	4.19	3.950	3.950	1.499	1.70	6.0	0.1880
3.25	3.98	4.23	3.975	3.975	1.625	1.70	7.0	0.2271
3.50	4.00	4.25	4.000	4.000	1.752	1.70	8.0	0.2693
3.75	4.04	4.30	4.05	4.050	1.873	1.65	11.0	0.3599
4.00	4.10	4.30	4.10	4.080	1.995	1.65	11.5	0.4566
4.25	4.15	4.35	4.15	4.156	2.119	1.65	13.0	-
4.50	4.20	4.40	4.20	4.18	2.243	1.65	14.0	0.6685
4.75	4.35	4.51	4.35	4.34	2.356	1.65	14.5	1.0135
5.00	4.49	4.63	4.50	4.50	2.4749	1.70	14.0	1.3753
5.25	4.71	4.91	4.75	4.74	2.5929	1.75	13.5	2.0093
5.50	5.52	5.65	5.50	5.50	2.710	1.80	12.8	3.9979
5.75	6.25	6.65	6.40	6.41	2.8398	1.85	12.0	6.4954
6.00	7.25	6.95	7.00	7.01	2.9722	2.00		3.2390

TABLE - 7.

$X = \log B + 2.611 \log h^{-1}$

$B_1 = -3.140X10^{-4}$ M (100 ppm. $Al_2(SO_4)_3$)

$B_2 = -4.71X10^{-4}$ M (150 ppm. $Al_2(SO_4)_3$)

$B_3 = -6.281X10^{-4}$ M (200 ppm. $Al_2(SO_4)_3$)

$B_4 = -7.852X10^{-4}$ M (250 ppm. $Al_2(SO_4)_3$)

$B_5 = -7.422X10^{-4}$ M (300 ppm. $Al_2(SO_4)_3$)

$\bar{n}$	X_1	X_2	X_3	X_4	X_5
0.25	6.8849	6.83905	6.80745	6.79985	6.8139
0.50	6.9371	6.86515	6.87270	6.87815	6.8922
0.75	6.9632	6.91735	6.91170	6.91469	6.9705
1.00	7.0415	7.00870	7.04235	6.99560	7.0227
1.25	7.1459	7.10005	7.06845	7.08250	7.1532
1.30	7.2110	7.16530	7.1337	7.1522	7.1923
1.50	7.2764	7.23055	7.25898	7.2566	7.2837
1.75	7.4330	7.4524	7.4263	7.40015	7.4142
2.00	7.6679	7.6220	7.6035	7.6615	7.6752
2.25	8.012	8.0527	7.9689	8.0045	8.0145
2.50	8.8729	8.9140	8.9346	8.8748	8.84970

APPENDI. II

Table IA Results of protein (Bovine serum albumin) titration

Ionic Strength - 0.01 (0.01 mKCl)

Bovine serum albumine conc. - 100 mg per litre

Total volume - 200 ml

pH(p)-pH of protein solution

pH(B)-pH of blank

ml HCl	pH(p)	pH(B)	ml HCl	pH(p)	pH(B)	ml HCl	pH(p)	pH(B)
0.0	6.25	6.015	2.3	2.900	2.885	6.1	2.540	
0.1	4.37	3.220	2.4	2.880	2.875	6.3	2.430	
0.2	4.03	3.900	2.5	2.865	2.855	6.5	2.420	
0.3	3.87	3.770	2.6	2.850	2.840	6.7	2.405	
0.4	3.77	3.635	2.7	2.835	2.825	6.9	2.390	
0.5	3.64	3.540	2.8	2.815	2.805	7.1	2.375	
0.6	3.535	3.430	2.9	2.795	2.790	7.3	2.360	
0.7	3.450	3.360	3.0	2.785	2.785	7.5	2.350	
0.8	3.390	3.300	3.1	2.760	--	7.7	2.340	
0.9	3.330	3.280	3.3	2.730	--	7.9	2.335	
1.0	3.290	3.240	3.5	2.710	--	8.1	2.320	
1.1	3.260	3.200	3.7	2.685	--	9.0	---	2.310
1.2	3.210	3.180	3.9	2.655	--	9.1	2.260	

Table IA (contd.)

ml HCl	pH(p)	pH(B)	ml HCl	pH(p)	pH(B)	ml HCl	pH(p)	pH(B)
1.3	3.165	3.150	4.0	--	2.665	10.0	--	2.220
1.4	3.130	3.080	4.1	2.630	--	10.1	2.215	
1.5	3.105	3.000	4.3	2.610	--	11.0	---	
1.6	3.075	3.000	4.5	2.595	--	11.1	2.175	
1.7	3.050	2.970	4.7	2.570	--	12.1	2.130	
1.8	3.020	2.990	4.9	2.555	--	13.1	2.100	
1.9	3.000	2.990	5.1	2.530	--	14.1	2.070	
2.0	2.970	2.960	5.3	2.515	--	15.1	2.035	
2.1	2.950	2.940	5.5	2.500	--	16.1	2.010	
2.2	2.920	2.910	5.7	2.480	---	16.6	2.000	
			5.9	2.460				

Table 1B

pH(p)	$\log CH_1$	$\log CH_2$	$\log[CH_1-CH_2]$
4.37	-4.220	-4.370	-4.7540
4.03	-3.900	-4.003	-4.5007
3.87	-3.770	-3.870	-4.4572
3.77	-3.635	-3.770	-4.2083
3.64	-3.540	-3.640	-4.2269
3.535	-3.430	-3.535	-4.0930
3.450	-3.360	-3.450	-4.0878
3.390	-3.300	-3.390	-4.0252
3.330	-3.280	-3.330	-4.2434
3.290	-3.240	-3.290	-4.2041
3.260	-3.200	-3.260	-4.0880
3.210	-3.180	-3.210	-4.3556
3.165	-3.150	-3.165	-4.6192
3.130	-3.080	-3.130	-4.0438
3.105	-3.00	-3.105	-3.6680
3.075	-2.97	-3.075	-3.6372
3.020	-2.99	-3.020	-4.1675
3.000	-2.990	-3.000	-4.6383
2.970	-2.960	-2.970	-4.6198

Table 3 Al(III)-Protein (Bovine serum albumin) Interaction

Ionic Strength - 0.01 [0.01 M KCl]

Aluminium conc.- 9×10^{-4}M [Aluminium Sulphate]

Bovine serum albumin conc. - 100 mg per litre

Total volume - 200 ml

pH(p)-pH of protein + aluminium solution

pH(B)-pH of only aluminium solution

ml NaOH 0.1 M	pH(p)	pH(B)	ml NaOH 0.1 M	pH(p)	pH(B)	ml NaOH 0.1 M	pH(p)	pH(B)
0.0	3.77	4.25	2.4	4.290	4.30	4.7	4.560	4.830
0.1	3.85	4.295	2.5	4.300	4.30	4.8	4.590	4.950
0.2	3.92	4.300	2.6	4.310	4.30	4.7	4.620	5.120
0.3	4.02	4.300	2.7	4.320	4.31	5.0	4.650	5.280
0.4	4.09	4.290	2.8	4.330	4.320	5.1	4.710	5.675
0.5	4.12	4.290	2.9	4.330	4.335	5.2	4.780	6.095
0.6	4.16	4.280	3.0	4.34	4.350	5.3	4.830	6.350
0.7	4.20	4.280	3.1	4.35	4.365	5.4	4.190	6.635
0.8	4.23	4.275	3.2	4.355	4.375	5.5	5.115	6.875
0.9	4.24	4.270	3.3	4.36	4.390	5.6	5.435	7.275
1.0	4.255	4.270	3.4	4.36	4.405	5.7	5.630	7.515
1.1	4.270	4.270	3.5	4.37	4.425	5.8	5.905	7.750
1.2	4.270	4.270	3.6	4.385	4.440	5.9	6.175	7.980

Table 3 (Contd.)

ml NaOH 0.1 M	pH(p)	pH(B)	ml NaQH 0.1 M	pH(p)	pH(B)	ml NaOH 0.1 M	pH(p)	pH(B)
1.3	4.275	4.270	3.7	4.40	4.455	6.0	6.550	8.145
1.4	4.230	4.270	3.8	4.415	4.475	6.1	6.750	8.270
1.5	4.255	4.270	3.9	4.420	4.495	6.2	6.990	8.445
1.6	4.265	4.275	4.0	4.440	4.515	6.3	7.370	3.520
1.7	4.260	4.280	4.1	4.450	4.550	6.4	7.530	3.600
1.8	4.282	4.280	4.2	4.460	4.600	6.5	7.620	3.650
1.9	4.280	4.230	4.3	4.480	4.620	6.6	7.320	8.700
2.0	4.280	4.280	4.4	4.495	4.665	6.7	3.02	8.755
2.1	4.230	4.285	4.5	4.510	4.705	6.8	--	8.800
2.2	4.280	4.290	4.6	4.540	4.765	6.9	--	8.830
2.3	4.290	4.295	--	--	--	7.0	--	8.360

Table 4 - Al(III)-Protein(casein) Interaction

Ionic Strength - 0.01 (0.01 M KCl)

Aluminium conc.- 9×10^{-4}M[Aluminium Sulphate]

Casein Conc. - 100 mg per litre

Total volume - 200 ml

pH(p)-pH of (protein + aluminium) solution

pH(B)-pH of aluminium solution

ml NaOH 0.1M	pH(p)	pH(B)	ml NaOH 0.1 M	pH(p)	pH(B)	ml NaOH 0.1 M	pH(p)	pH(B)
0.0	3.88	4.250	2.4	4.290	4.320	4.5	4.590	4.670
0.1	3.93	4.285	2.5	4.305	4.325	4.6	4.620	4.720
0.2	3.98	4.310	2.6	4.305	4.335	4.7	4.660	4.775
0.3	4.035	4.330	2.7	4.310	4.400	4.8	4.725	4.885
0.4	4.080	4.310	2.8	4.320	4.350	4.9	4.815	4.980
0.5	4.145	4.310	2.9	4.335	4.360	5.0	4.850	5.100
0.6	4.150	4.310	3.0	4.340	4.365	5.1	4.935	5.530
0.7	4.155	4.310	3.1	4.340	4.370	5.2	5.000	5.795
0.8	4.200	4.300	3.2	4.350	4.385	5.3	5.150	6.270
0.9	4.210	4.300	3.3	4.365	4.390	5.4	5.430	6.540
1.0	4.230	4.300	3.4	4.375	4.410	5.5	5.740	6.850
1.1	4.240	4.300	3.5	4.385	4.420	5.6	6.020	7.230
1.2	4.255	4.300	3.6	4.400	4.425	5.7	6.560	7.455

Table 4 (contd.)

ml NaOH 0.1 M	pH(p)	pH(B)	ml NaOH 0.1 M	pH(p)	pH(B)	ml NaOH 0.1 M	pH(p)	pH(B)
1.3	4.260	4.300	3.7	4.415	4.445	5.8	6.660	7.665
1.4	4.270	4.300	3.8	4.425	4.460	5.9	6.555	7.845
1.5	4.270	4.300	3.9	4.450	4.485	6.0	7.385	7.925
1.6	4.270	4.300	4.0	4.475	4.510	6.1	7.570	8.050
1.7	4.270	4.300	4.1	4.495	4.525	6.2	7.770	8.175
1.8	4.270	4.300	4.2	4.505	4.560	6.3	7.905	8.270
1.9	4.270	4.300	4.3	4.535	4.590	6.4	8.070	8.370
2.0	4.270	4.300	4.4	4.560	4.630	6.5	8.280	8.450
2.1	4.275	4.310	--	--	--	6.6	8.365	8.500
2.2	4.280	4.315	---	--	--	6.7	8.475	8.550
2.3	4.285	4.320	--	--	--	6.8	8.600	8.600
--	--	--	--	--	--	6.9	8.690	8.670
--	--	--	--	--	--	7.0	--	8.700